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Adenofibrosis in the Rat Liver

With Persistence of Polychlorinated Biphenyls in Adipose Tissue

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Fifty male Sherman strain rats were fed 500 ppm of a polychlorinated biphenyl (PCB) (Aroclor 1254) for six months. Five each were killed zero, one, two, three, four, six, eight, and ten months after exposure to Aroclor had ceased. The livers of these rats were examined by light and electron microscopy. Liver lesions persisted although exposure to PCBs ceased.

Ten months after exposure ceased, 1,192 ppm PCBs were still present in the rats' adipose tissue and 22.65 ppm in the rat livers.

Aroclor patterns found in the tissues by electron capture gas chromatography differed from patterns of dietary Aroclors. Mass spectral analysis of liver and adipose tissue revealed three major Aroclor components with masses of 324, 358, and 392. These contained isotopic clusters indicative of the presence of Cl_5, Cl_6, and Cl_7, respectively.

In a previously reported study[1] Sherman strain rats were fed polychlorinated biphenyls (PCBs) (Aroclor 1254 and Aroclor 1260). The rats developed characteristic morphological changes in the liver. These changes consisted of hypertrophy of the liver cells, a brown pigment in Kupffer cells, lipid accumulation in the cytoplasm of hepatocytes and, at the higher dietary levels, adenofibrosis. Because of the wide distribution of PCBs in the environment[2] and their persistence, the hepatic lesion was studied further. The present study was undertaken to see whether, once exposure to PCBs was stopped, the morphological changes produced in the liver might disappear.

Materials and Methods

A total of 50 male weanling, specific-pathogen-free, random-bred Sherman strain rats were tagged individually and group-caged, ten rats per cage. They were given Aroclor 1254 in their diet for six months. The dose of 500 ppm, which is equivalent to an average daily intake of 36.4 mg/kg body weight,[1] was chosen because in the previous study all ten exposed male rats developed adenofibrosis within a period of eight months. The Aroclor was mixed into ground laboratory chow as previously described.[1] After six months of

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Fig 1.—Area of adenofibrosis in liver section consisting primarily of ducts surrounded by flattened epithelium. Ducts are surrounded by little fibrosis (hematoxylin-eosin, original magnification × 150).

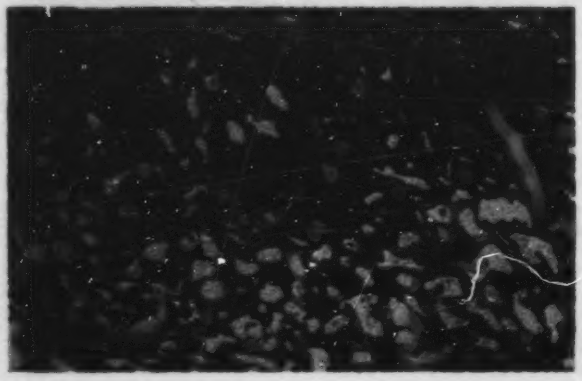

Fig 2.—Rat liver section showing cluster of small columnar cells resembling pancreatic tissue adjacent to blood vessel. Hepatocytes surrounding lesion are vacuolated (hematoxylin-eosin, original magnification × 175).

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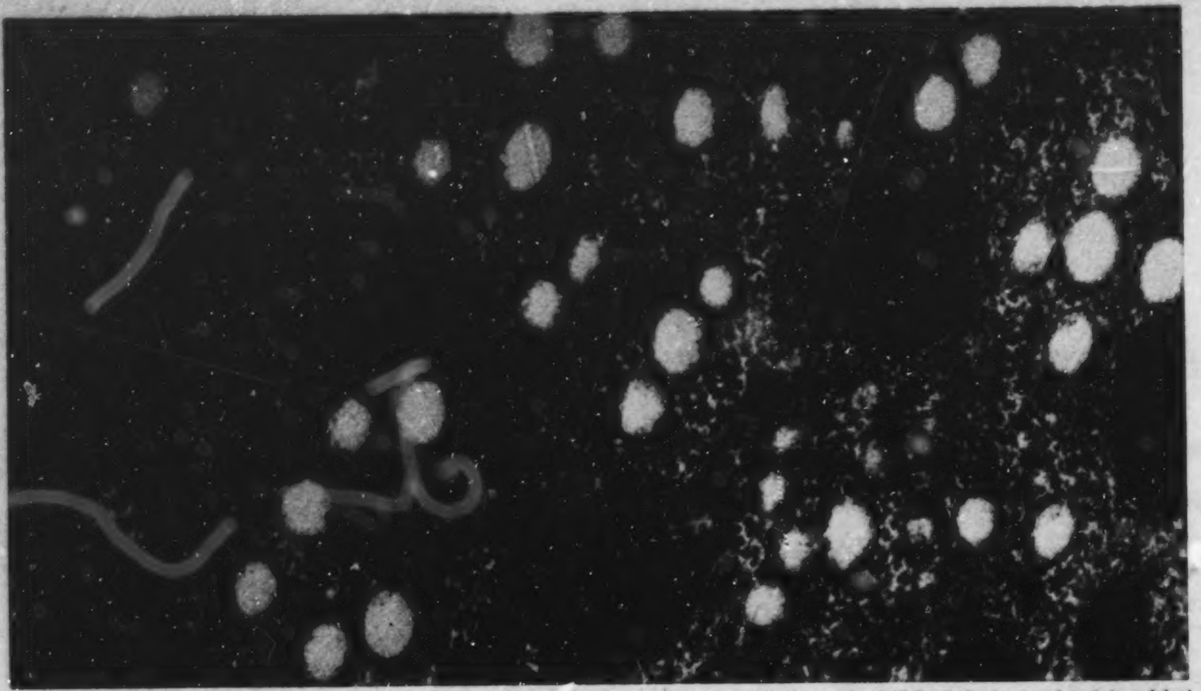

Fig 3.—Section of hepatic tissue in latter part of recovery phase illustrating numerous small lipid vacuoles (LV) (lead citrate-uranyl acetate, original magnification × 51,300).

Fig 4.—Portion of hepatocyte with tubules of endoplasmic reticulum (ER) arranged in disorderly fashion. N represents nucleus; M, mitochondria (lead citrate-uranyl acetate, original magnification × 20,235).

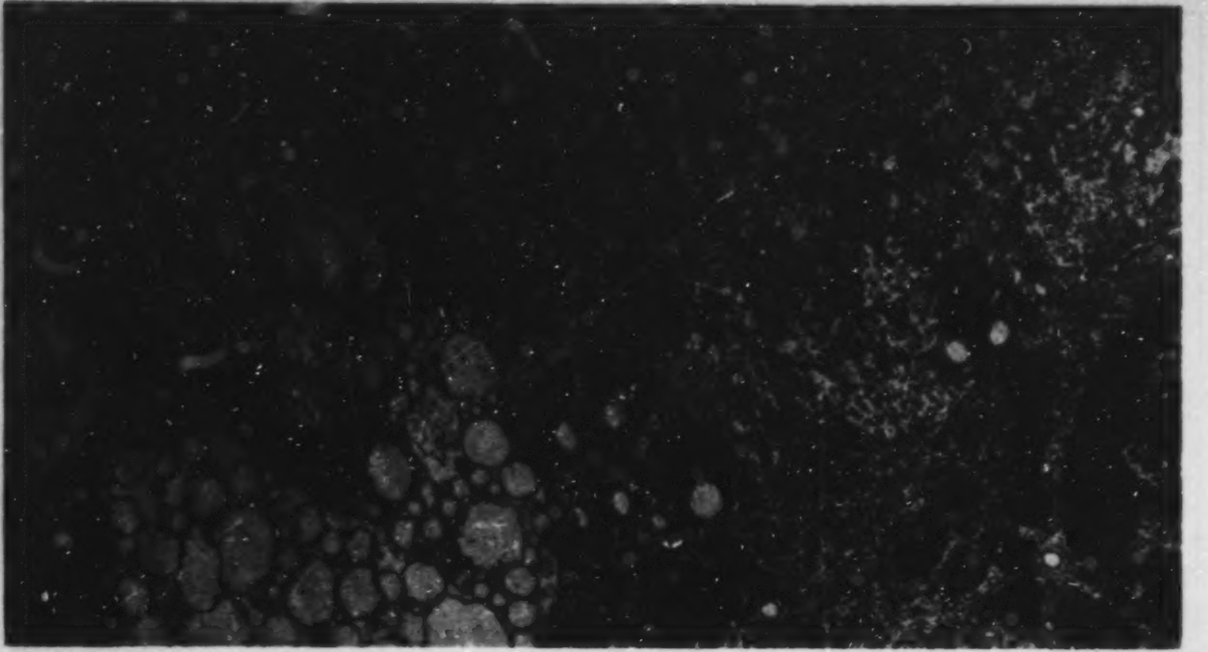

Fig 5.—Border of area of adenofibrosis in liver. Note round clusters of dark granules intermixed with web-like clusters of vacuoles. These formations illustrate appearance of brown pigment (BP). C represents collagen; LC, liver cell (lead citrate-uranyl acetate, original magnification × 20,235).

receiving the experimental diet, the rats received no more Aroclor and were fed plain laboratory chow. At this time five rats were killed. Additional killings of five randomly selected rats were made one, two, three, four, six, eight, and ten months after exposure to PCB had been discontinued. At autopsy the livers were removed, weighed, and the tissue fixed in phosphate-buffered 5% glutaraldehyde for three hours for electron microscopic examination, post-fixed in 1% osmium tetroxide, embedded in maraglass, sectioned with a glass knife, and stained with lead citrate and uranyl acetate. Tissue for the light microscope was fixed in 4% buffered formaldehyde solution and stained with hematoxylin and eosin. Frozen tissue of selected formalin-fixed hepatic tissue was cut on the cryostat and stained with oil red 0 to demonstrate lipids. At the time of the last sacrifice, ten months after exposure to PCB-containing diets had been discontinued, adipose tissue and liver from seven previously exposed rats and the adipose tissue and liver from two rats of the same age that had been fed only plain chow were analyzed for the presence of PCBs by electron capture (EC) gas chromatography according to methods described by Curley et al.[3]

In order to determine which Aroclor constituents were of such chemical stability that they remained at a measurable residual level ten months after dietary intake had ceased, adipose and hepatic composites were analyzed by gas chromatography-mass spectrometry. The conditions for mass spectral analysis were as follows: the mass spectrometer was interfaced with a gas chromatograph and equipped with a mass marker accurate within ±0.3 mass units. The gas chromatographic column temperature was 220 C; flash heater, 235 C; glass-coiled column, 3.05 m × 0.64 cm; 1.5% OV-17/1.95% QF-1 on 60/80 mesh chromosorb "W" (high performance, acid washed, dimethyldichlorosilane treated), helium pressure; 0.84 kg/sq cm; flow rate (rotameter setting 3), 45 ml/min; separator temperature, 225 C; source energy, 70 ev; accelerating voltage 3.5 kv; trap current 60μamp; box current 50μamp, and leak current 8μamp.

Results

During the course of the experiment three rats died. The livers of the rats that were killed at the time exposure to Aroclor 1254 was discontinued were enlarged, with average weights of 6.32% of the body weight. When the experimental diets had been discontinued for four months, the liver made up only 3.6% of the body weight, and after eight months recovery the average weight amounted to 3.34% of the average body weight. The fraction of the body weight for the liver in control adult male rats ranges from 2.44% to 2.59%.[1]

Forty livers were studied microscopically. The hepatocytes were enlarged in 39 livers and all 40 livers showed either vacuolated or foamy cytoplasm because of increased lipid accumulation. Cytoplasmic inclusions similar to those described previously[4] were observed in the livers of 24 rats. Adenofibrosis, which in many instances was very extensive, was present in 36 of the 40 livers, and a brown pigment was noted in the Kupffer cells and other macrophages in 25 livers. Of the four rats that did not develop adenofibrosis, one each was killed two and six months after the poisoned diets were discontinued. The other two rats without adenofibrosis were killed ten months after onset of exposure. The adenofibrosis ranged in size from grayish-white lesions measuring 0.5 to 1 cm to areas of 5 cm with a pitted surface. Quite often the lesion was multicentric. The adenofibrosis[1] is illustrated in Fig 1 and 2. In this study the appearance of

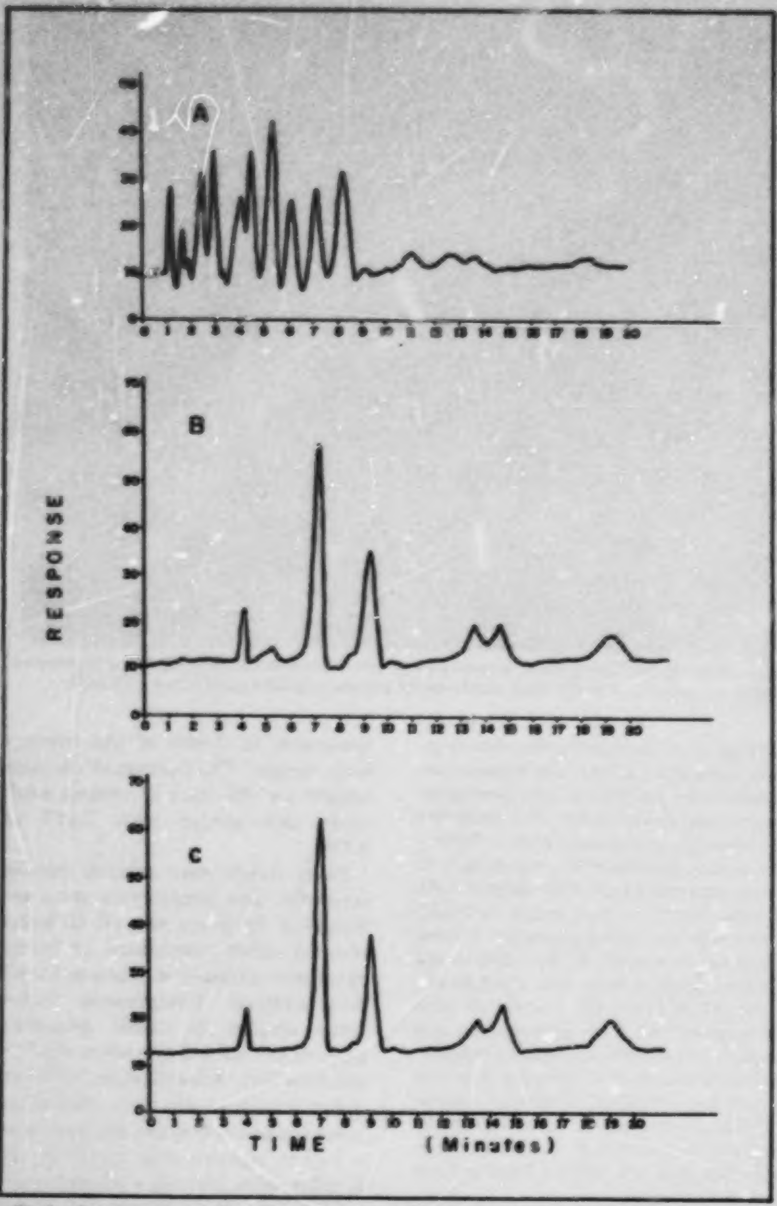

Fig 6.—Gas chromatographic traces of Aroclor 1254 standard (A), hepatic tissue extract (B), and extract of adipose tissue (C). Response expressed as percentages, with 100% = 1.0 mv.

the lesion, particularly when it was extensive, varied a great deal. Small lesions consisted predominantly of glandular pale-staining epithelial cells that formed ducts and were surrounded by very little fibrosis. The larger lesions had extensive fibrosis and contained collagen, and often the ducts which were formed by the epithelial pale-staining cells were markedly dilated. The dilated ducts contained mucus or necrotic debris, and at times formed small cysts measuring up to 0.5 cm in diameter. Small dilated ducts were lined by columnar epithelium, and larger ones by flattened epithelium (Fig 1).

In areas of extensive fibrosis, and also adjacent to blood vessels in areas of normal hepatic parenchyma (Fig 2), clusters of small glandular cells were seen in 15 of the livers which resembled the epithelium observed normally in the pancreas. These small clusters of pancreatic-like tissue have not previously been described in the livers of rats or of other species. Special stains for esterase, esterase with sodium taurocholate, and tryptophan showed a positive reaction indicative of salivary gland tissue. The significance of these cells is presently not understood. Normally tissue of a pancreatic type is not observed in the livers of our rats. Clusters of small columnar epithelial cells resembling pancreatic tissue also occurred in portal triads without being surrounded by typical adenofibrosis. In the present study, one to two rats at each killing showed these small columnar cells with highly acidophilic cytoplasm and a small, round nucleus.

In the older animals, the fibrosis was quite extensive in some instances and the glandular proliferation was present mainly at the periphery of the lesion. Signs of regression of the lesion were not noted, except for the disappearance of epithelial cells from the center of the lesion. Furthermore the lipid accumulation and the hypertrophy of the hepatic cells remained constant throughout the study. Extensive areas of necrosis were observed in three of the 40 livers.

In addition to the change described previously,[1] electron microscopic examinations of sections of liver tissue showed numerous small lipid vacuoles in the cytoplasm of many hepatocytes, particularly in the latter part of the recovery phase (Fig 3). The smooth and rough endoplasmic reticulum was arranged in a disorderly fashion (Fig 4). Many of the mitochondria were atypical and surrounded by double membranes.

The electron microscopic appearance of the brown pigment present in the macrophages and Kupffer cells is illustrated in Fig 5. Large clusters of dark granular material, intermixed with lipid vacuoles, were observed, as well as round clusters of granular material about the size of mitochondria. In our previous studies it was found that at least some of this pigment contained iron[1] but in addition high concentrations of uroporphyrin (75% 8-carboxyprophyrins and 25% 7-carboxyprophyrins) were found in the livers,[5] which could account for some of the pigment. In addition to these findings, oil red O stain demonstrated granules of red-staining lipid material in the area of the brown pigment.

Since the morphological changes in the liver did not disappear, the remaining seven rats were killed after a ten-month recovery phase and their adipose tissue and livers analyzed for Aroclor. In adipose tissue, the concentrations of PCBs ranged from 924 ppm to 1,686 ppm, with an arithmetic mean of 1,192 ppm. The concentrations in the liver ranged from 17.30 ppm to 26.24 ppm, with an arithmetic mean of 22.65 ppm. The PCB levels in control adipose and liver tissues were <1.0 ppm.

The gas chromatogram obtained from the EC analysis of adipose tissue did not differ from that of liver, but was significantly different from standard Aroclor 1254. An average of seven peaks was observed as a residue in the tissues. Under these gas chromatographic conditions, at least 18 peaks have been observed in the standard material (Fig 6). The difference is gas chromatographic patterns between stored and ingested Aroclor has been observed before in this laboratory and by other researchers in the field.[6,7]

The total ion current (TIC) chromatogram for the liver tissue composite is shown in Fig 7. Mass spectral analysis revealed the presence of only three major Aroclor components with masses of 324, 358, and 392. These components contained isotopic clusters indicative of the presence of Cl_5, Cl_6, and Cl_7, respectively. Adipose tissue analysis by mass spectrometry revealed the same patterns. Although only three different molecular components were present, examination of the EC and TIC chromatograms coupled with measured intensities from multiple scanning of eluting peaks indicates that stereoisomers of these three molecules are obviously present. Peaks 1 to 3 (Fig 7) did not contain any chlorinated moieties, while other peaks observed in the liver TIC contained the following molecular ions: peak 4 (324); peaks 5 to 7 (392 and 358); and peaks 8 to 9 (392).

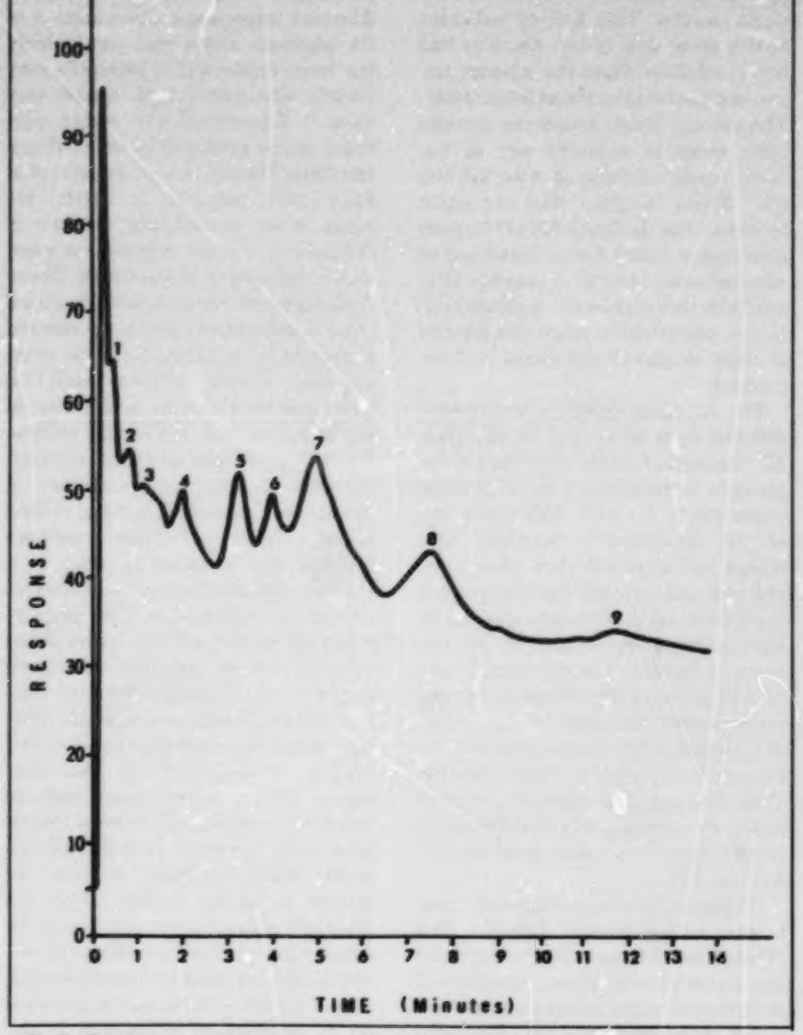

Fig 7.—Total ion current chromatogram of sample of composite liver. Response expressed as percentages, with 100% = 10.0 mv.

Comment

The adenofibrosis of the liver produced in this study and the one previously reported[1] is the result of Aroclor consumption. Whether the Aroclor itself, or a contaminant such as a chlorinated dibenzofuran, is responsible still needs to be resolved.

A study of the lesion shows that epithelial proliferation occurs in the periphery, while the central portion of the lesions contains primarily fibrous tissue. The present study does not completely resolve the question of reversibility of this lesion since the storage levels of PCBs in adipose tissue and liver may have been sufficiently high to stimulate continued growth of the altered hepatic tissue. The levels in the liver suggest that a constant low-level turnover of PCBs takes place in the rats. In a previous study, Curley et al[3] found levels of 10,000 ppm Aroclor 1254 in adipose tissue of rats that had been fed 500 ppm of the mixture in their diet for eight months. This finding indicates that a great deal of the Aroclors had been mobilized from the adipose tissue and excreted in the present study. The storage levels found ten months after onset of recovery are in the same range of those of rats fed 100 ppm PCBs in their diet for eight months.[3] The dietary level of 100 ppm produces a much lower incidence of adenofibrosis,[1] and it is possible that adenofibrosis represents a permanent lesion, particularly since the percent of body weight of the livers had decreased.

The Aroclors found in the tissues differed from those fed in the diet. Metabolism of lower chlorinated biphenyls is considered as a possible explanation for the differences observed analytically between consumed and stored PCB. Earlier work of Block and Cornish[8] has shown that biphenyls and chlorobiphenyls can be hydroxylated and excreted in the urine of rabbits. A more recent study by Hutzinger et al[9] indicates that the rat is capable of hydroxylating mono-, di-, and tetrachlorobiphenyls, but not hexachlorobiphenyls. Since Aroclor 1254 averages five chlorines per molecule, it is conceivable that the heavier chlorinated moieties could remain as a residue.

P-dimethylaminoazobenzene also produces adenofibrosis. Edwards and White[10] and Bennett et al[11] described the lesion in rats when a mixture of chlorinated naphthalenes and chlorinated diphenyls were fed. Unfortunately, the finding was not well described in Bennett et al's report, but Edwards and White mentioned Bennett et al's finding as another example of the induction of adenofibrosis by chemicals in an excellent description of the lesion. A critical review of the histopathogenesis of this lesion was made by Stewart and Snell[12].

The presence of the brown pigment in Kupffer cells and macrophages is of interest and was to a lesser extent also observed in the earlier study.[1] Electron microscopic examination of the pigment shows that particularly the large clusters of it resemble very closely illustrations of ceroid pigment.[13] Experimentally ceroid pigment can be produced by hemorrhage into fatty tissues rich in unsaturated fatty acids, particularly in the absence of an antioxidant (vitamin E deficiency). Ceroid pigment is commonly referred to as lipofuscin. These lipid pigments are at times associated with hemosiderin and may contain iron-positive granules as in the present case.[1] Ceroid granules were also described by Edwards and White in the livers of rats fed butter yellow. Bennett et al also observed a brown pigment in rats fed a mixture of chlorinated naphthalenes and chlorinated biphenyls. These authors claimed that it failed to stain in a manner characteristic of either hemosiderin or hemofuscin. The porphyrins demonstrated in the livers of our rats fed Aroclor may also contribute to the overall pigment deposits. Ceroid pigment increases in people with age and has therefore been called "*Abnutzungspigment*" by the Germans. Attempts have been made to relate it to senility. It has been associated with neuronal ceroid-lipofuscinosis (Batten disease) in man. In minks, a disease called yellow fat disease[14] is associated with the accumulation of excessive amounts of ceroid in adipose tissue. The accumulation of ceroid in mink can be prevented by addition of vitamin E to the diet. It occurs when the food rations contain high percentages of fish scrap. However, Gorham et al suggest that other factors may also be involved, since high dietary percentages of fish scrap did not promote growth as well as the stock ration, even when vitamin E was added to the diet. These observations raise the question whether high doses of vitamin E, and possibly the addition of choline to the diet, could prevent or minimize the observed effects of PCBs on the liver of our rats.

Richard L. Moore assisted with the animal tests.

Linda W. Anderson assisted with preparation and interpretation of the electronmicrographs.

Annie R. Alford performed the tissue preparations.

H. L. Stewart, MD, and the tissue laboratory of the National Cancer Institute, Bethesda, Md, performed evaluations of liver slides.

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PUBLICATION

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Health Hazards of Discarded Pesticide Containers

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The proper disposal of various types of "empty" pesticide containers continues to be a problem in agricultural areas and in some public health vector control projects. These discarded containers constitute a potential health hazard, mainly to children. The seriousness of this hazard is in direct relation to its propinquity to areas frequented by children.

These so-called "empty" containers are never completely empty. Johnston reported a fatal poisoning case involving a 9-month-old child who played with a discarded can containing a pasty mixture of DDT and parathion.[6] Johnston also cited another incident in which 2 children were found playing with sacks that had contained 25% water-wettable powdered parathion. One of these children developed mild symptoms of poisoning and had a depressed blood cholinesterase level. The other child showed no signs or symptoms of poisoning. McGee et al. described the fatal poisoning of a child who played in a shed covered with metal sheets made from a drum that had contained toxaphene.[7]

The present paper describes a study to evaluate the more important hazards associated with discarded containers. Smoke from burning paper bags was analyzed for parathion content, and "empty" bags and drums were tested for residual parathion. Methods for decontaminating drums were evaluated, and an attempt was made to determine a practical, quick method to render these discarded drums nonhazardous until they could be destroyed.

Methods

Experience has shown that the most hazardous containers are those which have held one of the more toxic organic phosphorus compounds such as parathion. In the Pacific Northwest, parathion is usually sold as a 25% water-wettable powder or 1% or 2% dust in paper bags, while the most common liquid formulation is the 45.6% emulsi-

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fiable concentrate which is usually sold in 5-gallon metal drums.

Samples of smoke from burning parathion bags were collected by means of an air-sampler arrangement consisting of two 500-ml. Greenburg-Smith impingers in series with a column containing glass wool, glass beads, and activated alumina. The impingers collected particulate matter, and the column was designed primarily to collect vapor. Each impinger contained 250 ml. of ethyl alcohol. The intake tube was held in the smoke, and the air sample was drawn through the impinger and column. Vacuum was provided by an electrically-driven pump. Each sample represented 4 cu. ft. of air which was drawn through the sampler in 15 to 25 minutes. The air volume and time were accurately measured for each sample.

Parathion content of air samples and other samples was determined by the method of Averell and Norris.[3]

Results

Observation on Disposal of "Empty" Pesticide Containers.—In orchard or row-crop areas of the Pacific Northwest where large amounts of pesticides are used, observations indicate that most applicators destroy burnable containers at regular intervals during spraying or dusting operations or immediately after the operation is completed at a particular location. This procedure requires only the minimal effort and time to arrange containers in a pile and strike a match. The person performing the operation usually stands by to make sure all containers are completely burned. It has been observed that very few of these individuals make any special effort to avoid breathing the smoke.

Disposal of metal or glass pesticide containers is more difficult and time-consuming and has not been as prompt as the disposal of burnable containers. These metal or glass pesticide containers are usually left at the loading site of the spray machine until some later date at which the operator will have time to remove them.

Glass containers are usually broken and buried or at least broken when taken to a public dump ground. If, instead of being destroyed, they are left in the orchard or on the farm, they may become particularly hazardous, especially if the poison warning label has been lost through exposure to moisture. Orchard laborers often collect one-gallon glass jugs for use as containers for drinking water. In one search by the authors among "empty" pesticide containers, a one-gallon jug was found which contained 5 ml. of 20% TEPP (tetraethyl pyrophosphate). If taken orally, this amount alone would probably be enough to kill an average adult. Such a container would require considerable decontamination before it could be safely used for drinking water.

Since metal cans and drums are difficult to crush or bury, they are usually left somewhere on the farm, or, if aerial application is involved, they may be left in a pile at the flight strip. As many as 60 such drums have been found piled at one flight strip used by aerial applicators. In orchard areas, as many as a dozen drums can sometimes be found on a particular farm at one time. After a large number of such containers have accumulated, they may be hauled to a public dump or landfill where scavenging persons or landfill workers may come in contact with them or may use them as containers or for some other purpose. The 5-gallon drum is usually of sturdy construction and looks particularly desirable to the farmer as a container for oil or some other liquid material being used on the farm. As a result, he has a tendency to keep 2 or 3 of the more recently emptied drums on hand.

Itinerant orchard workers sometimes use empty 5-gallon metal pesticide drums as stools in their orchard dwellings. As with the glass jugs, the labels on these containers may be lost through exposure to weather, and it is possible that these drums may be used by persons not aware that they had contained poisons.

Parathion Content of Discarded Paper Bags and Metal Drums.—Determinations were made of the amount of residual parathion in 12 paper bags that each had contained 4 pounds of 25% parathion water-wettable powder. The amount found ranged from 1.0 to 4.8 gm., with a mean of 2.5 gm. of the 25% material. In terms of technical parathion, the range was 0.25 to 1.20 gm. and the mean was 0.60 gm.

Twenty-two discarded 5-gallon drums representing 3 different pesticide manufacturers were obtained from an airplane applicator. These drums had contained 45.6% parathion emulsifiable concentrate. After use they had been left in the open near a hangar, piled not more than 2 drums deep with at least a large portion of the top of each can exposed. All had vent holes punched in the top, and only 2 had caps replaced on the pouring spouts. On top of each drum was 2 or 3 inches of snow. Each drum contained some ice water, presumably from rain or melting snow.

These drums were taken to the laboratory where the outside of each was washed and the contents thawed and measured. The contents appeared to be primarily water, mostly very rusty and dirty and in some cases milky. In 3 drums a small amount (15 to 70 ml.) of what appeared to be undiluted emulsion remained. The quantity of liquid varied from a few milliliters that were hardly measurable to 6,020 ml. The concentration of parathion found in these liquids varied from 0.03% to 0.22%, and, based on the volume of liquid, the various drums contained from 1.25 to 9.48 gm. (mean 2.73 gm.) of technical parathion in suspension. The color developed in the analysis was typical of parathion and did not indicate any significant concentration of *p*-nitrophenol.

Four of these drums were chosen at random, after excluding those which were nearly dry when found. Each was rinsed 6 times with alcohol and the rinsings were analyzed for parathion content. The 4 drums contained 7.14, 8.07, 8.51, and 14.63 (mean 9.59) gm. of residual technical parathion, respectively, as estimated by 6 alcohol rinses. Four rinses removed approximately 95% of the total residual parathion from each drum. The average total parathion content in these drums was 12.32 gm., made up of 2.73 gm. in liquid form and 9.59 gm. residual which was removed by alcohol rinse.

Parathion Content of Smoke from Burning Pesticide Containers.—Six air samples were taken from the smoke of burning paper bags which had contained 25% water-wettable parathion. The results indicated that the smoke contained from 4.4μg. to 12.1μg. of parathion per liter, with a mean of 7.9μg. per liter. The parathion that was collected by the impingers represented 77.3% of the total amount recovered. The remaining 22.7% was collected by the column of alumina, glass beads, and glass wool.

Evaluation of Methods of Decontamination of Empty Metal Pesticide Drums. Tests were conducted to determine the value of strong alkali as a means of decontaminating drums that had contained parathion emulsion. Parathion is generally thought to be hydrolyzed rapidly in strongly alkaline solutions. Gunther and Blinn reported that 50% hydrolysis of parathion occurs in 1 normal alkali in 32 minutes.[8] Peck states that 50% hydrolysis of parathion in saturated lime water requires 8 hours.[9]

In preliminary tests carried out in laboratory glassware using solutions of 45μg of parathion per milliliter of saturated lime water, a minimum of 22 hours was required to hydrolyze half of the parathion under conditions of continuous agitation at room temperatures. In other tests without agitation and in some with an excess of hydrated lime, 50% hydrolysis required up to 4 days. Hydrolysis in distilled water was negligible over a period of 17 days. A commercial preparation of sodium hydroxide and other ingredients designed to clean out clogged drains (Drano*) was tested in an aqueous system containing 4.8% Drano and 0.009% parathion. This material hydrolyzed over 50% of the parathion in 2 hours but had not hydrolyzed as much as 90% in 20 hours. When the concentration of parathion was increased stepwise up to 0.09%, the rate of hydrolysis was decreased.

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* Manufactured by Drackett Co., Cincinnati. The use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service.

To simulate the sort of decontamination operation which might be practical under field conditions, one pound of hydrated lime was added to each of 2 "empty" parathion drums, and sufficient tap water was added to fill them. The solutions were agitated with an electrical stirring apparatus for 10 minutes and then allowed to stand at room temperature. Samples were withdrawn periodically for analysis. Just before sampling the solution in the drum was again agitated for 10 minutes. After 16 days, the concentration of parathion in these drums had decreased less than 50%.

Although both of the chemical agents (lime and Drano) used were somewhat effective in decontamination, the time and expense involved in their use and the problem of disposal of the relatively concentrated alkali solutions would probably prevent their widespread acceptance by farmers, orchardists, or commercial spray operators.

In fruit orchard areas it has been observed that spray machine operators, when using emulsifiable concentrates, rinse empty pesticide drums only once, and then usually with only a small amount of water. In some cases they are not rinsed at all. In light of these observations, tests were conducted to determine the amount of decontamination which can be produced by a more thorough water rinsing.

Sixteen 5-gallon and 3 one-gallon metal drums which had contained parathion concentrate were obtained from an open-air disposal area. These containers had been opened, vented, emptied, and allowed to remain uncovered for an unknown period of time. None were thought to be more than from 4 to 6 months old. Visual inspection indicated they contained very little or no water.

All cans were rinsed at least 3 times with water. Six cans were rinsed 4 times, and 1 can was rinsed 5 times. The water volume used per rinse was 3,840 ml. for the 5-gallon-size drum and 1,000 ml. for the 1-gallon-size. Rinsings were poured into wide-mouth glass jars, and aliquots were immediately taken for analysis for parathion content.

The results of these analyses are shown in the Table. Although these drums were supposedly empty, it should be noted that rather large quantities of parathion were recovered in the first wash from every drum. These amounts ranged from 3.0 to 33.3 (mean 12.5) gm. of technical parathion per gallon of capacity. These amounts could easily cause the death of children or adults if the contaminated cans were improperly handled. After the second rinse, very little parathion remained in the drums, as shown by the fact that the third rinse removed only about 1% of the total removed by 5 rinses. This rinsing procedure would possibly be even more effective for removing residual parathion from freshly emptied drums than from the weathered drums used in this test.

Comment

From observations made in the orchards of north central Washington, it would appear that the major health hazards of discarded pesticide containers might be associated with exposure of workers to the smoke from burning paper bags and with exposure of children to the residual pesticide left in discarded metal drums and other containers.

The parathion content of the smoke from burning paper bags (7.9 mg. per cubic meter) was significantly higher than that found in air during spraying operations. Batchelor and Walker reported that the average concentration of parathion in air during orchard spraying ranged from 0.03 to 0.09 mg. per cubic meter depending on the formulation and method of application used.[2] These workers reported higher average parathion concentrations (0.53 to 0.76 mg. per cubic meter) during mixing and loading. When evaluating the relative hazards involved, however, one should keep in mind that orchard spraymen customarily are

Parathion Content of Water Rinses from "Empty" Metal Drums That Originally Contained Parathion Concentrate

Parathion Content of Concentrate (%)	Capacity of Drum (gal.)	No. Drums Tested	Value	Parathion Content First Rinse (Gm/Gal Capacity)	Second Rinse (Gm/Gal Capacity)	Third Rinse (Gm/Gal Capacity)	(% of Total)	Fourth Rinse (Gm/Gal Capacity)	Fifth Rinse (Gm/Gal Capacity)
25	5	1	Range Mean ± S.E. *	-- 8.4	-- 0.80	-- 0.20	-- 2.7	-- --	-- --
42	5	8	Range Mean ± S.E. *	8.6—33.3 14.8± 2.9	0.20—5.6 1.10±0.7	0.03—0.16 0.09±0.03	0.3—1.0 0.6±0.1	0.05—0.06 † 0.06±0.004	-- --
45.6	5	5	Range Mean ± S.E. *	3.3—21.6 8.0± 3.4	0.20—0.7 0.40±0.1	0.09—0.19 0.14±0.02	0.7—3.8 2.6±0.7	0.07—0.13 ‡ 0.10±0.03	-- 0.15
47.4	5	2	Range Mean ± S.E. *	4.3—27.5 15.9±12.2	0.10—0.9 0.50±0.4	0.01—0.14 0.08±0.06	0.2—0.5 0.4±0.02	-- 0.02	-- --
47.4	1	3	Range Mean ± S.E. *	3.0—25.9 13.2± 0.7	0.05—0.11 0.09±0.002	-- 0.01	0.1—0.3 0.2±0.1	-- --	-- --
Total	--	19	Range Mean ± S.E. *	3.0—33.3 13.5± 2.0	0.05—5.6 0.60±0.3	0.01—0.28 0.10±0.02	0.1—3.8 1.1±0.3	0.02—0.13 0.06±0.01	-- 0.15

* Standard error of the mean.
† Based on 3 cans tested.
‡ Based on 2 cans tested.

exposed for an entire workday of 8 hours or more, while a worker would only be exposed to smoke from burning bags for a short time. The concentration of parathion detected in the smoke (7.9 mg. per cubic meter) also exceeds the 0.1 mg. per cubic meter value set by the Committee on Threshold Limits, American Conference of Governmental Industrial Hygienists as the maximum level to which workers should be exposed for an 8-hour working day.[5] The possibility that heat or light from the fire might transform parathion into paraoxon or other more toxic metabolite was not evaluated in the work reported here.

Based on the measured parathion content of the smoke (7.9 mg. per cubic meter or 7.9μg. per liter) and assuming a tidal air volume of 1,740 L. per hour during the light work[4] of burning paper bags, it may be calculated that the inhalation of parathion is at a rate of 13.7 mg. per hour. Thus, if a worker should stand in the smoke for a period of 5 minutes while waiting for a pile of bags to burn, he could be exposed through the respiratory route to 1.15 mg. of parathion. This value may be compared to the respiratory exposure during orchard spraying with parathion which has been estimated to be between 0.16 and 0.19 mg. per hour.[3]

In addition to farmers who might be exposed occupationally and children who might play with empty containers, another group of persons who have contact with discarded pesticide containers are workers at garbage dumps and landfill areas. Mail et al. studied the exposure to insecticides of employees at a city landfill.[5] The results of chemical analyses on blood, urine, alcoholic handwashes, and exposure patches indicated that the exposure of these employees to parathion was minimal. Mail and his coworkers concluded that disposal of containers for organic phosphorus insecticides at a city landfill could be done with safety to personnel when recommended precautions were followed. These authors were primarily concerned with containers and waste material from plants manufacturing and formulating pesticides rather than with containers emptied during field use of pesticides. For the safety of scavenging persons who might be tempted to use the containers, empty pesticide containers should be consigned to a landfill only if immediate crushing and covering of the containers as soon as they are delivered can be assured.

From the results reported above, it appears that the use of strong alkalis for decontamination of empty parathion drums is not effective, and, additionally, the cost involved in time and material would make the procedure impractical. However, since only a small amount of parathion was found in the third rinse and in subsequent rinsings, it seems that rinsing twice with water removed a major fraction (almost 98%) of the emulsifiable parathion in the drums. Additional water rinsings appeared to have little effect.

Thus, 2 thorough rinsings with water should render used parathion drums significantly safer than unwashed containers. Each rinsing should involve pouring approximately one gallon of water into a 5-gallon drum and shaking it vigorously for approximately 20 shakes. These rinsings could be added to the spray tank before it is filled to final volume without significantly affecting concentration. Even though adequate rinsing reduces the hazard of empty metal drums left on the premises after spray operations, it should not be considered a substitute for eventual destruction of the containers by crushing and burying them.

If spray operators are not able to destroy metal containers soon after use, it is suggested that they at least expend enough effort to rinse these containers more thoroughly as soon as they are emptied. Educating operators to this practice may not be particularly difficult if they are led to realize that the mere use of an adequate amount of rinse water and the completion of 2 rinsings would mean the difference between leaving a hazardous container and leaving one in which it would be difficult for children to find enough residual toxic pesticide to cause much harm. This practice involves only one additional rinsing and the use of a greater volume of water in comparison to what is usually done by many spray operators, and therefore would require only about 2 minutes additional time per drum. In an orchard spray operation where only 2 to 4 drums are used per day, this would mean only 4 to 8 extra minutes per day for safety's sake.

Summary

Observations made in orchard and row-crop areas of the Pacific Northwest indicate that most applicators destroy burnable containers soon after their content of pesticide has been emptied. Disposal of glass or metal containers, and particularly those of metal, is less prompt. Occasionally, these glass or metal containers may be used as drinking-water jugs or for other household uses.

Residual parathion in 12 paper bags that had each contained 4 pounds of the 25% water-wettable powder was found to range between 0.25 and 1.20 gm. with a mean of 0.60 gm. of the technical material. Twenty-two 5-gallon metal drums which had contained 45.6% parathion emulsifiable concentrate were found to contain residual amounts of technical parathion ranging between 1.25 and 9.48 gm. with a mean of 2.73 gm.

Air samples were taken in the smoke from burning paper bags that had contained 25% water-wettable parathion. Analyses indicated that the smoke contained from 4.4μg. to 12.1μg. of parathion per liter with a mean of 7.9μg. per liter.

Tests indicated that strong alkali solutions were not satisfactory for decontaminating 5-gallon metal drums which had contained parathion. Rinsing such containers twice with water removed almost 98% of the total removable parathion, as estimated by 5 water rinses.

It is recommended that 2 rinsings with water be used as a minimum decontamination for 5-gallon metal drums after their content of pesticide has been removed. In addition, the drums should eventually be made unusable and destroyed by crushing and burying them.

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INSECTICIDE ACTIVITY IN MAN

Urinary Excretion of Paranitrophenol by Volunteers Following Dermal Exposure to Parathion at Different Ambient Temperatures

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and W. V. HARTWELL

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INSECTICIDE ACTIVITY IN MAN

Urinary Excretion of Paranitrophenol by Volunteers Following Dermal Exposure to Parathion at Different Ambient Temperatures

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The rate of absorption of parathion, as reflected by urinary excretion of paranitrophenol, was measured in human volunteers exposed to 2% parathion dust at different ambient temperatures. Rates of paranitrophenol excretion varied directly with temperature between 58° and 105° F. Maximum excretion occurred 5 to 6 hours following initiation of exposure. Neither depressions in cholinesterase activities nor clinical symptoms were observed following the exposures used.

THE ORGANIC phosphorus insecticide parathion is a useful material for controlling insects of economic and public health importance. It has a high toxicity to humans, however, and each year deaths are attributed to its use. In an effort to understand more fully the toxic hazards associated with parathion, studies have been made of factors that influence the mechanisms and rates of absorption. Two methods have been used to measure parathion absorption. They are determination of activities of cholinesterases, enzymes that are inhibited by parathion, and quantitation of a urinary metabolite, paranitrophenol (*3*).

Excretion of paranitrophenol and depression of cholinesterase activities reflect different physiologic functions. Changes in enzyme activity ordinarily cannot be determined by the Michel method following brief light exposures, because normal variations in the method are greater than the changes produced by the compound (*1*). Paranitrophenol assay is useful under these conditions however, since microgram quantities can be measured and the metabolite is absent from the urine of unexposed individuals. The quantity of paranitrophenol excreted indicates the magnitude of absorption and, less directly, the magnitude of exposure, but it does not necessarily measure the physiologic effects of the exposure. Changes in cholinesterase activities reflect physiologic effects resulting from parathion.

A direct quantitative correlation between changes in enzyme activities and paranitrophenol excretions has not been

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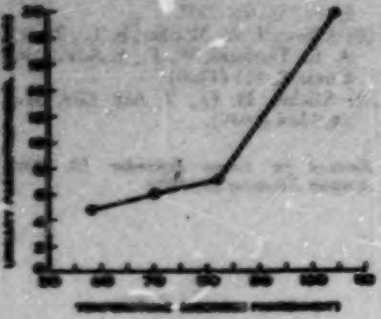

Figure 1. Average hourly rates at which p-nitrophenol was excreted in urine for 41 hours following dermal exposures sustained at different temperatures to 5 grams of 2% parathion dust by three volunteers

Table I. Paranitrophenol Excreted by Human Volunteers Following Dermal Exposures to 2% Parathion Dust at Different Ambient Temperatures

Hours after Start of Exposure	Paranitrophenol Excreted, µg. per Hr.							
	58° F.^{a}		70° F.^{a}		82° F.^{a}		105° F.^{a}	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
1–2	4.1–5.4	4.8	3.7	3.7	5.3–13.5	8.2	15.8–135.8	59.7
3–4	6.1–8.9	7.5	22.3	22.3	5.3–17.0	10.4	15.8–135.8	69.8
5–6	4.0–15.4	9.7	25.0	25.0	18.8–24.2	21.9	48.2–155.3	88.6
7–8	2.4–4.0	3.2	15.1	15.1	14.5–18.3	16.4	17.8–106.9	66.1
9–10	2.0–6.9	5.4	13.7	13.7	12.8–19.6	16.9	13.2–69.2	35.9
11–15	6.3–7.2	6.7	12.0	12.0	7.8–15.0	11.4	12.2–38.3	20.3
16–20	4.5–6.7	6.6	10.2	10.2	3.7–8.9	5.7	4.8–27.4	13.2
21–30	1.6–2.1	1.8	5.2	5.2	2.3–2.6	2.4	4.1–11.7	7.5
31–40	1.7	1.7	1.9	1.9	2.3–6.2	3.4	2.5–6.9	4.8
41+	...	...	1.2	1.2	2.1–4.0	3.0	1.5–4.3	3.2

^{a} Two exposures were sustained at 58° F., one at 70° F., three at 82° F., and nine at 105° F.

demonstrated in humans under controlled conditions. Most of the available information on this relationship was obtained from studies of persons suffering occupational exposure under conditions that precluded precise measurement of the duration of exposure, quantities of toxicant per unit area of body surface, and temperature (*1*). This report presents data on sequential excretions of paranitrophenol and activities of cholinesterases in blood obtained from human volunteers exposed percutaneously to parathion dust under controlled conditions.

Procedure

Each of four volunteers sustained dermal exposures at different temperatures after 2% parathion dust was applied to the right hand and forearm. Volunteers were exposed at weekly or less frequent intervals at 58°, 70°, 82°, and 105° F. The order of exposure was selected at random. Two individuals sustained exposures at 105°, 82°, and 58° F.; one at 105° and 70° F.; and one at 105° F.

For each exposure, 5 grams of 2% parathion dust was placed in a polyethylene bag wide enough to accommodate the hand and long enough to cover the hand and forearm to the elbow. The formulation used was a commercial preparation of 2% parathion on pyrophyllite, the actual chemical content of which was not determined. The hand and forearm were secured in the bag with masking tape, and the toxicant was distributed as evenly as possible over the exposure area. The hand and forearm, still in the plastic bag, were then placed in a constant temperature chamber, and the chamber port was secured with rubber damming and masking tape. Exposures continued for 2 hours. To ensure only dermal exposure, the subjects breathed from a source of pure compressed air while being placed in the exposure situation and during decontamination. Following the 2-hour period, the hand and arm were decontaminated by scrubbing the exposed area with soap and water and a hand brush for 5 minutes. This was followed by two washings in ethyl alcohol contained in a polyethylene bag.

Urine was collected as individual voids for 40 or more hours following exposures. Voids were kept separate, and the time and date recorded on each sample bottle so that hourly excretion rates could be determined for each void. Intervals varied from 30 minutes to 8$^1/_2$ hours. Each sample was assayed for paranitrophenol by the method of Elliott *et al.* (*3*). Average excretion rates were calculated at 2-hour intervals for the first 10 hours following exposure. This was done by dividing the total paranitrophenol excreted during each period by the time interval corresponding to the void. Rates were determined at 5-hour intervals for the next 10 hours and at 10-hour intervals for the remaining period.

Determinations for cholinesterase activity were made on blood samples taken prior to the first exposure, following decontamination, and thereafter for 4 days. Since no significant change in enzyme activities was noted, fewer blood samples were taken in connection with later exposures. Red blood cell and plasma cholinesterase activities were determined by the micro-Michel procedure (*4*).

Results

Data on paranitrophenol excreted by human volunteers who sustained discrete dermal exposure to 2% parathion dust at different ambient temperatures are presented in Table I. Maximum paranitrophenol excretion rates occurred 5 to 6 hours following initiation of exposures within temperature range 58° to 105° F. As the exposure temperatures were increased, a progressive increase in the rate of paranitrophenol excretion was observed, although other exposure conditions remained constant.

The average total excretion of paranitrophenol at different temperatures was: 58° F., 196 µg. during 40 hours; 70° F., 246 µg. during 41 hours; 82° F., 287 µg. during 41 hours; and 105° F., 804 µg. during 41 hours. These values permitted calculation of hourly rates used to plot the line in Figure 1, which shows a direct relationship between exposure temperature and the mean values for hourly rates of excretion of paranitrophenol on all exposures sustained at each temperature. The same relationship is indicated by mean values at the 5- to 6-hour time interval of Table I.

Red blood cell and plasma cholinesterase values were measured on 12 samples taken before exposures and on 11 samples following exposures. Mean value for RBC cholinesterase before exposure was 0.49 ΔpH per hr., standard deviation 0.247; after exposure, mean RBC value was 0.51 ΔpH per hr., standard deviation 0.264. Mean pre-exposure value of plasma cholinesterase was 0.74 ΔpH per hr., standard deviation 0.56; and mean postexposure value was 0.73 ΔpH per hr., standard deviation 0.55. These data indicate no significant changes in enzyme activities following these exposures. Under the conditions of the study, no positive relationship was demonstrated between cholinesterase activity and paranitrophenol excretion. A possible explanation for this observation may be that the largest excretion recorded over a 41-hour period was 1.2 mg. of paranitrophenol, corresponding to 2.5 mg. of parathion. Edson (*2*) reported that volunteers tolerated daily oral doses of 3 mg. of parathion without cholinesterase depression or clinical effect.

Although no significant changes were noted in cholinesterase activities and no clinical signs of poisoning were observed during or following exposures, local effects attributed to contact with paresthesias were observed at the highest temperatures. For several days following decontamination, more perspiration was noted on the exposed area than elsewhere on the body.

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Diagnostic Problems in Toxicology (Agriculture)

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TOXICOLOGY PROGRAM
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The chief basis for correct diagnosis in toxicology is a history of adequate exposure to a chemical, associated with the presence of characteristic signs and symptoms. When both a clear history and clear signs are present, such a diagnosis is among the easiest in medicine. When either the history or signs are unclear or even lacking, such a diagnosis can be extremely difficult.

Sometimes the patient, or those who accompany an unconscious patient, are simply unaware of an exposure which has occurred; or they may be aware of an exposure but completely uninformed about its significance. In such cases, a high index of suspicion on the part of the physician may lead to a correct diagnosis. In a recent case,[26] a child suffered a sudden seizure and was brought quickly to a hospital where he was found to be limp, unresponsive, and slightly cyanotic. The pupils were widely dilated and the pulse "very slow." The total absence of reflexes and a history of a fall from his tricycle just before onset suggested to a neurosurgeon that injury to the child's brain stem had occurred. Atropine was given because of excess fluid in the respiratory tract. The child's tolerance for moderate doses of atropine without atropinization and the fact that his father was a spray pilot aroused suspicion that the youngster might be poisoned by an organic phosphorus insecticide. Although the father denied this possibility, a dose of 250 mg. of 2-pyridine aldoxime methiodide (2-PAM) was administered. The child recovered to near normal in less than 20 minutes. Parathion was recovered from the feces and paranitrophenol from the urine. It was shown that the blood cholinesterase, which had been severely depressed, was returned rapidly to near normal by the drug. It turned out that the child, who suffered from pica, had eaten dirt contaminated by parathion. It was established that spillage of the parathion had occurred more than 6 months earlier. Soil from the place where the child had dug still contained nearly 1% of parathion.

A totally different sort of problem is presented by the patient who gives a history of trivial exposure and complains of illness that is not known to be characteristic of poisoning by the compound involved. These cases are among the most troublesome and time-consuming that the toxicologist encounters. Some of these cases result from honest mistakes. The associates of a person who has really been poisoned may suffer so much apprehension for his own safety that he requires treatment, although he is not really poisoned. People who happen to be near a fire or explosion where chemicals are present may suffer similar apprehension. For example, in Pomona, Calif., an insecticide warehouse burned several years ago. It was clear from the clinical account which we received that many of the people who were treated suffered from fright rather than intoxication. After some of them were treated with small doses of atropine, they complained of dryness of the mouth and other discomfort, a thing which does not happen to people given large doses of the drug as a treatment for poisoning by one of the organic phosphorus insecticides.

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People who are frightened by real accidents usually recover promptly. The same is not true of those who attribute their vague, recurrent, or chronic disease to minimal or even nonexistent exposure to chemicals. Many of these people have had their self-diagnosis directed and reinforced by irresponsible statements in the medical and the lay press that have been reviewed elsewhere.[18] These articles have alleged that insecticides, and especially residues of them in foods, are the cause of many human and animal diseases. Thus, DDT has been claimed to be the direct cause of a virus disease (later shown to be influenza), a psychoneurotic syndrome, and a contributing cause of poliomyelitis, hepatitis, cardiovascular disease, and cancer as well as several diseases of animals. Other claims have attempted to relate insecticides with fungus infection, allergy, sinusitis, gastrointestinal upsets, dehydration, alkalosis, malnutrition, pneumonitis, and insanity. The difficulty is that the responsible toxicologist, knowing that the manifestations of poisoning in man may differ from those in animals and knowing that there are wide variations in individual susceptibility, feels a continuing responsibility to winnow the chaff for the small amount of grain it may hold.

Statistics on Poisoning by Pesticides

As already mentioned, a physician should have a high index of suspicion regarding the possibility of poisoning. Information on the actual occurrence of cases may be useful for guiding this suspicion. Most poisoning by pesticides falls in the category of accidental poisoning by solids and liquids. Poisoning by fumigants forms only a small proportion of the category of accidental poisoning by gases and vapors. For over 20 years, the death rate in the United States associated with accidental poisoning by solids and liquids has varied from 0.8 to 1.2 per 100,000 population. In recent years, it has usually been 0.9 per 100,000 population. This represents a considerable improvement over rates of 3.4 to 5.2 recorded[30] between 1900 and 1909. Conley[16] reported that 7.8% to 12.8% of poisoning cases each year from 1946 to 1955 were caused by pesticides. The value found by Short[30] in an especially careful study of the 1956 statistics fell within this range. Thus, the death rate from pesticides in the United States is approximately 0.09 per 100,000. Of the 152 deaths caused by all pesticides in 1956, 104 were caused by compounds introduced before DDT—54 attributed to arsenic alone. Only 35 deaths definitely were associated with new synthetic compounds, although some of the 13 other deaths caused by unidentified pesticides were probably caused by synthetics. Of the 35 deaths, 16 were caused by organic phosphorus insecticides. During 1956, children up through the age of 9 years accounted for 94 deaths from pesticides (62%); 78, or just over one-half of the total number, were in children 3 years old or younger. These figures, similar to those reported by Conley[16] for 1946 to 1955, suggest that it is most important to consider poisoning in connection with children and important to consider the older pesticides, as well as the newer ones, in every case of suspected poisoning.

Guides for Diagnosis

Information on the older pesticides usually adequate to permit diagnosis of poisoning by them is easily available. One of the best sources is Sollmann's *Manual of Pharmacology.*[39] Another extremely useful volume is that by von Oettingen entitled simply *Poisoning.*[48]

Information on the newer pesticides is somewhat less available. Facts on some formulations may be found in the popular volume by Gleason, Gosline, and Hodge entitled *Clinical Toxicology of Commercial Products.*[26] The constituents of a much larger number of formulations may be found in the latest annual edition of the *Pesticide Handbook*, edited by Frear.[23] A description of the medical aspects of selected pesticides may be found in the booklet, *Clinical Memoranda on Economic Poisons,* issued by the Communicable Disease Center.[16]

There is a tendency to ignore the contribution of solvents in the production of poisoning. Information on these materials may be found in Browning's book, *Toxicity of Industrial Organic Solvents*[25] and also in von Oettingen's treatise on *Halogenated Hydrocarbons, Toxicity and Potential Dangers.*[41]

The Toxicology Section of the Communicable Disease Center is responsible, within the Public Health Service, for research on the toxicology of pesticides. Inquiries about diagnostic problems are given as much attention as our resources permit, and may even give rise to a full-scale research project. I would like to invite all of those interested in the toxicology of pesticides to send appropriate samples of blood, tissue, excreta, or stomach contents to us. It is hoped that significant results will be published either by those who send samples or by mutual authorship.

Specific Topics in Diagnosis

Chiefly because of their potential for causing acute injury, the organic phosphorus compounds have received much attention. Measurement of cholinesterase activity of the blood is a widely used laboratory test for establishing a diagnosis of poisoning by these compounds or for screening workers to detect those whose exposure has been excessive, in order to prevent the occurrence of poisoning. However, many laboratory personnel, physicians, and persons in industry who use the test are not familiar with the difficulties of its interpretation. It is generally known that a "true" acetylcholinesterase occurs in the red blood cells while a so-called "pseudo" cholinesterase occurs in the plasma. Both may be measured by a variety of laboratory procedures, of which the method of Michel[34] is recommended. Quite a number of laboratories are now able to carry out this procedure accurately. Both the true and the pseudo cholinesterases occur in nervous tissue where the true acetylcholinesterase is responsible for transmission of an important proportion of nerve impulses. The measurement of these enzymes in nervous tissues and other solid tissues is carried out generally by manometric techniques usually available in research laboratories only. A number of surveys reviewed by Ganelin[34] have shown that there is a wide range of normal variation in blood cholinesterase levels. Rider et al.[37] found, in a study of 400 healthy men and 400 healthy women, the values shown in the accompanying table. With insignificant exceptions, the values found by other investigators have fallen within the ranges shown there.

Cholinesterase Activity (Δ pH/Hr) of Normal Human Blood

	Men	Women
Red Cells, range	0.53-1.02	0.34-1.10
mean	0.766	0.750
Plasma, range	0.44-1.64	0.26-1.54
mean	[illegible]	0.817

In addition to the variation between individuals, there is also great variation in enzymatic activity in the same person over a period of time. Published studies of this variation[19,28,31] have shown maximal fluctuations from 13% to 25% for red cell enzyme and from 20% to 23% for plasma cholinesterase. Studies done in the Toxicology Section have shown even greater variability. It is, therefore, clear that a decrease in enzyme activity of 30% may indicate normal variation only, while a much smaller decrease in another situation could be significant of anticholinesterase effect. Thus, it has been shown by a number of authors that mild poisoning can occur in the presence of blood cholinesterase levels within the range of normal.[30,40] Really severe poisoning is apparently always associated with a severe depletion of blood cholinesterase levels. However, a similar severe depletion of blood enzyme activity is frequently seen in asymptomatic workers who have had repeated exposure to parathion.[24,40] The same could undoubtedly occur with any of the highly toxic organic phosphorus insecticides. The rate at which cholinesterase is depleted is just as important as the degree of depletion in determining whether illness will result.

Sufficient injury to the liver can interfere with production of plasma cholinesterase by that organ. Fortunately, this effect is seldom seen to a significant degree except in patients who are sick enough to be hospitalized with obvious liver disease.

Despite its limitations, the measurement of cholinesterase activity is the only generally available test for organic phosphorus effects; and organic phosphorus exposure is one of the few factors affecting cholinesterase activity, particularly that of the red cells. When a decrease of 25% or more from preexposure activities is found in routine determinations in an apparently healthy person exposed to an organic phosphorus insecticide, one can logically assume that the depression is due to the insecticide and that the worker's safety technique or equipment requires study. Although this is the only unequivocal contribution of a routine cholinesterase test, it is felt that there is great value in such surveillance. In the absence of related clinical findings, a worker who shows a moderate depression of cholinesterase activity should be allowed to continue his work, as long as proper safety technique can be insured. If cholinesterase activity continues to decrease, one must assume that the subject is unable to follow safety regulations and he must be removed from contact with toxic materials. Also, within the limits stated, a determination of cholinesterase activity is useful in establishing a diagnosis of clinical poisoning. The decision as to when a person who has been poisoned should return to work must be made by the physician only after evaluating the severity of the illness, the length of exposure, the rapidity of return of cholinesterase activity toward normal, and the intelligence and reliability of the patient and his employer in regard to safety precautions.[24]

Recently, a greatly improved method for measuring paranitrophenol in urine has been developed.[20] This method permits the measurement of excretory products derived from parathion and other compounds which yield paranitrophenol on hydrolysis. At least in the case of malathion, it is possible to relate the excretion of organic phosphorus in the urine with dosage.[20,35] Although these measurements of excretion are valuable research tools, it is already clear that the results are subject to physiological variation just as are the results with cholinesterase. Following exposure to parathion, there is a broad correlation between the excretion of paranitrophenol and the occurrence of illness. However, in individual cases, more may be excreted by persons who remain well and less by persons who become sick. It is hoped that further study will at least define, if not fully explain, the variation.

Finally, as suggested by the case cited above, one of the oximes, and especially 2-PAM, may be used for a therapeutic trial in the diagnosis of severe illness that may represent poisoning by one of the common organic phosphorus insecticides. Poisoning by octamethyl pyrophosphoramide (schradan) and a few other compounds does not respond. The new drug has been used chiefly in cases involving parathion. Response has been truly dramatic and side-effects from the drug have been negligible. A review of this interesting development will soon be available.[19] The use of atropine in therapeutic trial is already recognized.

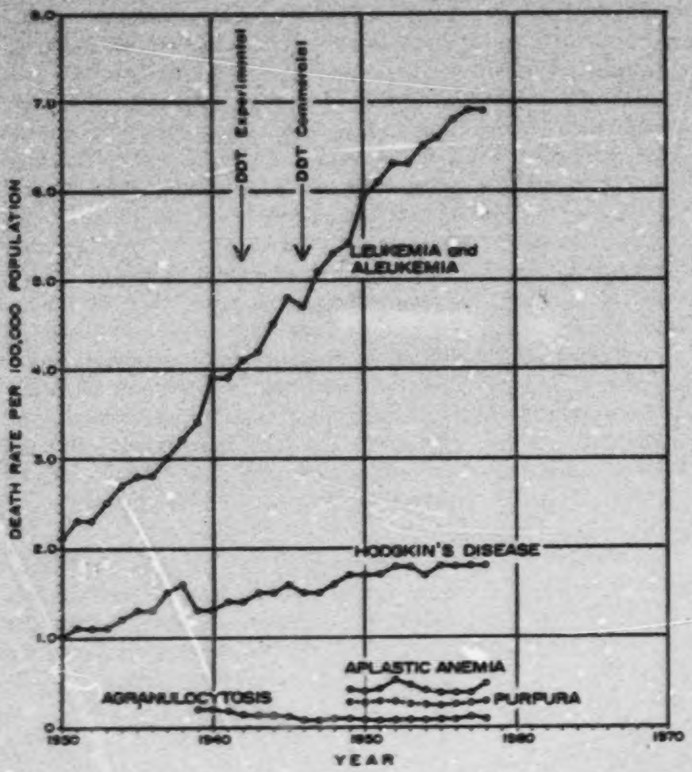
Diagnosis of poisoning by the chlorinated hydrocarbon insecticides is generally possible on the basis of history and symptomatology, but it may be assisted by measurement of the poisons or their metabolites in the tissues or the excreta. The signs and symptoms of poisoning are described in the references cited earlier. A word is needed here regarding the interpretation of chemical analyses. Because of the stability of these compounds, it is generally possible to demonstrate them in cases of acute poisoning. Stomach contents, as well as tissues, should be obtained if possible. The demonstration of one of these compounds in stomach contents is presumptive evidence that it was the cause of illness. However, the contribution of solvents and other ingredients in the formulations must not be forgotten. A major difficulty occurs in interpreting the presence of one of these insecticides in the tissues, or the excretion of a derivative in the urine. It has recently been shown that the distribution in the tissues is very different following a single dose as compared with repeated small doses. Following a single dose, distribution is relatively even in the different tissues, including the fat. In the course of repeated small doses, there is a gradual accumulation of these materials in neutral fat, accompanied by relatively small concentrations in the other tissues, including the blood. Moderate concentrations in the blood, brain, or viscera are consistent with a diagnosis of poisoning, while very much higher concentrations may be stored in adipose tissue in completely healthy people. Therefore, the history of exposure and/or the ratio of distribution of the chemical between the fat and the other tissues need to be taken into account in interpreting analytical results.

The organic mercury fungicides have caused severe poisoning in Sweden and some other places where they have apparently been used to a greater extent than in the United States. These fungicides are available in this country, and their action should be familiar to physicians who treat farmers and others concerned with the use of agricultural chemicals. The acute symptoms associated with irritation of the gastrointestinal system and renal failure caused by inorganic mercury compounds are seldom observed in poisoning by the organic mercury compounds. Instead, the nervous symptoms appear first, sometimes after months of latency. Early signs include fine tremors of the extended hands, loss of side vision, and slight loss of coordination, as in the finger-to-nose test. Incoordination is especially notable in speech and gait. Irritability and bad temper are frequently present, and the patient may complain of headache and other nonspecific dysfunction. Symptoms may follow relatively slight exposure and may appear as much as several weeks after exposure has ceased. Although there is some disagreement about the concentration of mercury that can be excreted safely, there is general agreement about the value of making the measurement. The most conservative view is that the concentration should not exceed 15μg. per liter.[9] Other investigators [18] who have presumably used more sensitive chemical methods, indicate that any operator excreting more than 50μg. of mercury per liter of urine should be placed on a "watch list" requiring weekly urinalysis and that any operator excreting more than 100μg. per liter be removed from exposure. The same authors warn that the excretion of mercury is irregular and individualized, following termination of exposure.

The differences in the toxic manifestations of the organic mercury compounds and the better known inorganic compounds is correlated with the greater storage of the organic compounds in the liver and kidneys [21] and also with the ability of some of the organic compounds to pass the blood-brain barrier.[27]

An Unsolved Problem in Diagnosis

The outstanding problem in diagnosis in toxicology is related to hypersensitivity. Although it is recognized that some of the pesticides (for example, pentachlorphenol and the chlorates) are direct irritants, it is the occasional case of hypersensitivity to a compound which causes no difficulty to the ordinary worker that taxes the diagnostic ability of the dermatologist. A more unusual, but also more acute problem relates to the blood dyscrasias. By sweeping generality the pesticides have been credited with causing an increase in blood dyscrasias. Actually, the available statistics for the United States indicate no increase in agranulocytosis, aplastic anemia, or purpura (Figure). During the period in which the use of pesticides in agriculture has increased, there has been an increase in Hodgkin's disease and a striking increase in leukemia and aleukemia. However, there



Death rates for selected causes, United States, 1930-1958. Source: *Vital Statistics of the United States,* each year. The years in which DDT was introduced experimentally and commercially are indicated. The other new pesticides were introduced at the same time as DDT or later.

has been no significant change in the rate of this increase since 1930. Certainly, there has been no accentuation of the increase associated with the introduction of DDT and the more recent introduction of other synthetic pesticides (Figure).

Only a small number of cases of blood dyscrasia of any sort have been reported in association with the modern pesticides. Apparently in no instance among these cases has the diagnosis been confirmed by giving the recovered patient a challenge dose of the pesticide suspected of causing the original disease and thereby precipitating a second attack. According to some investigators, this dangerous maneuver is the only certain way to establish that a specific chemical is the cause of a blood dyscrasia in a particular patient. Some dependence has been placed on patch tests, but these tests carry some danger and are not conclusive. As indicated above, the trends of incidence of the blood dyscrasias have not increased; however, this does not establish that pesticides are not the cause in individual cases. It is certainly agreed that chemicals, especially drugs, can be the cause of individual cases. It is also generally recognized that no really satisfactory diagnostic test is available for establishing the relationship between a particular chemical and a particular case of blood dyscrasia. I would like to make a plea for basic research in this field and draw your attention to one approach which has been taken.

It appears that the best way of demonstrating with reasonable certainty that a chemical is a cause of certain blood dyscrasias in a particular patient without further endangering the patient may be that involving in vitro tests with the patient's plasma. This matter has been discussed by Carr on page 389 et seq. of his review entitled "Drug Allergy."[14] The test, in whatever variation, has been best studied for thrombocytopenic purpura associated with a drug, Sedormid (allyl-isopropyl-acetyl-carbamide).[1-8] It appears that Sedormid combines with a protein in the platelets and probably in the endothelial cells of all patients to whom the drug is administered. Certain persons develop antibodies to the Sedormid-protein complex and thus become sensitive to the drug. If platelets from the sensitive person or from a normal person are exposed to Sedormid in saline and then brought in contact with the antibody from the patient's serum, the platelets will be agglutinated and, if complement is present, it will be fixed and the platelets will be lysed. Various aspects of the reaction may be demonstrated through complement fixation, through direct observation of agglutination and of lysis, and through observation of imperfect clot retraction in blood in which the platelets have been destroyed through lysis. It is believed that the injury to the endothelial cells occurs through a mechanism similar to that responsible for platelet destruction. Purpura of this etiology is dependent upon the injury to the endothelial cells but is accentuated by the thrombocytopenia.

The work with Sedormid has been confirmed to a considerable degree for thrombocytopenic purpura associated with quinine[26] and with quinidine.[10,11,22]

The results with agranulocytosis[17,25] do not agree closely with those for thrombocytopenia, but, even in the study of agranulocytosis, in vitro methods hold some promise. Furthermore, a complement fixation method has been explored with some success in connection with dermatosis caused by nickel.[44]

The in vitro tests discussed above have been applied to very few drugs and to no pesticides. Furthermore, the tests may never be applicable to certain forms of blood dyscrasia. Not much weight should be given to a negative result, but a positive result with appropriate controls is rather impressive. It may certainly be hoped that much more attention will be given to this matter, so that a really satisfactory test related to the ones just mentioned, or completely independent of them, may be developed. This would permit drugs and new chemicals which may cause blood diseases to be identifed clearly in individual cases.

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PESTICIDES PROGRAM
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INTERNATIONAL COOPERATION IN RELATION TO THE HAZARD OF PESTICIDES

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International cooperation in relation to the benefits of pesticides is well known. Most outstanding is work for the control and eventual eradication of malaria.

Less well known is cooperation in learning the dangers of chemical control and in developing safety measures that permit continued utilization of valuable compounds, both in agriculture and public health. Even in the technically advanced countries there are still problems associated with the manufacture, formulation, and especially the use of pesticides. These problems are accentuated in places where the potential dangers in the use of these poisons are not so well understood and their proper labeling is neglected.+

The most basic cooperation in science is that between individual scientists. It may involve the exchange of specimens, special instruments, and other things. But, most important, it involves exchange of information by formal publications, by letters, and by conversation. One way of bringing investigators from different countries together is meetings such as this one. Another way of getting investigators acquainted is an exchange of visits from one laboratory to another. These visits may be financed by the individuals themselves, by private foundations, by governments, by international agencies, or in other ways. Brief visits may be valuable and even lead to the exchange of views and cooperative effort over a period of years. More valuable are the exchanges of workers either as students or visiting lecturers in universities or as investigators in laboratories of different kinds. Finally, the international agencies of a single country (such as the International Cooperation Administration of the United States) or truly international organizations (such as the Pan American Sanitary Bureau and the World Health Organization) can finance and otherwise promote continuing cooperation for the solution of common problems, as they have already done so successfully in communicable disease control.

TOXICOLOGY OF DIELDRIN

The story of the toxicology of dieldrin will serve as an example of these various forms of cooperation. The first published account of occupational poisoning by dieldrin-related compounds came from the United States (1), but the report was not widely circulated. Earlier warnings about the possible hazard of using dieldrin (2) also failed to attract much attention.

Extensive occupational poisoning by dieldrin was first recognized in Venezuela by the staff of the Division de Malariologia, of which Dr. Arturo Berti is the Chief and Dr. Arnoldo Gabaldon is Counsellor. The initial studies were carried out principally by Dr. Jose Blazquez V. and Dr. C. A. Bianchini. Once these studies were made, a conference was organized in July, 1955, by the Division de Malariologia in cooperation with the Pan American Sanitary Bureau. The conference made it possible for the facts to become widely available even before publication (3). The conference was attended by representatives of the Venezuelan Ministry of Health, the Pan American Sanitary Bureau, the U.S. Public Health Service, the Mexican Antimalaria Project, and the manufacturers of the compound under discussion. A conference to discuss the implications of the new findings for other malaria control projects was called in Washington by the Pan American Sanitary Bureau in September, 1955. Additional studies were made by Blazquez and Bianchini (4).

Probably as an outgrowth of the first conference, Dr. G. J. Winthrop was attached to the Division de Malariologia. He and Dr. Jose R. Felice S. carried out a study (5) in which a number of others cooperated (6, 7, 8, 9). When this stage of the work was completed, a second Venezuelan conference was convened in May, 1957, in Maracay. This time there were investigators from England, as well as from Venezuela, Mexico, and the United States. A critical review of the information brought out by these conferences and by brief independent reports from Ecuador and Nigeria has been published elsewhere (10).

Because of the importance of dieldrin in the control of malaria, the study group convened by the World Health Organization in 1956 gave special attention to protective measures for its use (11). As a result of the interest of WHO, studies were made in Tanganyika (12) and in the United States (13) to measure the ex-

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posure of spraymen. Separate epistles of dieldrin poisoning in India were quickly recognized and ably reported both from a clinical and epidemiologic standpoint (14, 15).

Only shortly afterward the World Health Organization sent a toxicologist to study the relationship between spraying practices and the poisoning of workmen in several member countries using the compound for disease control (16). Evidence was assembled to show that the incidence of poisoning was proportional to intensity and duration of exposure and that other factors were not of critical importance. Experience in Indonesia showed that frequent bathing was not sufficient to prevent poisoning in the absence of other precautions. The extensive cooperative effort just outlined brought toxicologic information to dieldrin users in all parts of the world much faster than would have been possible otherwise.

Dieldrin is now used for malaria control in many countries. In Venezuela, where extensive occupational poisoning was first observed, careful application of pre-employment medical examinations and periodic surveys of safety measures, including the use of protective clothing, have resulted in an encouraging reduction in the incidence of poisoning (17).

However, the problem is not yet solved. Some cases continue to appear in spite of the precautions that have been possible. At the same time, dieldrin offers unique advantages, so that its use cannot be abandoned easily. A real hope for further improvement would lie in the use of any analytic method that would reflect dieldrin storage. It is well known that storage of a foreign compound eventually reaches equilibrium following continued dosage at a constant rate and that the level of equilibrium is proportional to the dosage rate. Under these circumstances, there is a proportionality between storage and excretion.

At the World Health Organization conference on pesticides held in Brazzaville in November, 1959, Fletcher (18) announced his success in measuring, in the form of organic chloride, a dieldrin-derived material isolated from the urine of spraymen. The subjects were provided with good protective clothing and required to practice good personal hygiene. No clear-cut evidence of poisoning had been detected in four years of spraying. The amount of dieldrin equivalent recovered from them represented from about 0.6 to 1.2 per cent of their daily dermal exposure, which had been measured earlier (12).

Finally, the diphenylamine-zinc chloride method developed by Cueto (19) for the chemical determination of dieldrin is both more rapid and more reproducible than the phenyl azide procedure available earlier. There is reason to think that it can be applied to dieldrin-derived material in human urine. Cooperative efforts are now underway to get and analyze samples of urine from spraymen who have been poisoned and from others who have remained well in spite of different degrees and durations of exposure. It is hoped that enough can be learned about the dynamics of dieldrin excretion to permit the use of a chemical test for the selection (and retirement) of spraymen who have been excessively exposed.

OTHER COOPERATIVE WORK

A number of current cooperative projects deserve mention. Following the suggestion of the study group referred to earlier, the World Health Organization issues every six months a *Bibliography on Pesticides, Toxicity, and Accidental Poisoning.* It also issues every six months an *Information Circular on the Toxicity of Pesticides to Man;* this publication deals primarily with accidents and measures to correct them. The World Health Organization offers by far the best institutional opportunity for the exchange of information in this field, whether the information is intended for formal publication or not. WHO offers financial aid and other support for research but maintains no laboratory of its own. Perhaps it may become practical for the World Health Organization to become more active than it now is in promoting the collection of toxicologic specimens and their distribution for study.

A number of other current needs should be listed. Among these is the need for more uniform nomenclature for widely used pesticides. Many countries have their own standards organizations, but one gets the impression that more uniformity of names would result if a "law of priority" were observed, as is done in the taxonomy of living things. Obviously, any attempts on the part of commercial companies to gain advantage by patents or other private use of recognized coined names is contrary to the interests of science.

Another continuing need is the one for a comparison of results from one laboratory to another. It is doubtful that methods should be standardized. Much is gained by variation of method, but this gain comes largely from attempts to correlate and explain the different results. A variant of this idea relates to recommendations and regulations. It would be a very difficult but perhaps rewarding task to compare the pesticide regulations that exist in different countries and correlate them with the safety records that are achieved.

In closing, I would like to invite all those interested in the toxicology of pesticides to send appropriate samples of blood, tissue, excreta, or stomach contents to the U. S. Public Health Service laboratory in Atlanta that is concerned with the mammalian toxicology of pesticides. It is hoped that significant results will be published either by those who send the samples or by mutual authorship. Such cooperation is becoming easier because, as a result of various contacts, many of those interested in the toxicology of pesticides are personal friends, whether they live in Bombay or Kerrville. Let us hope that this trend toward direct personal contact will continue.

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ATTENTION

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INSECTICIDE TOXICOLOGY

Manifestations of Cottonfield Insecticides In the Mississippi Delta

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A survey of school attendance and mortality records, hospital admissions, and illness among plantation groups was undertaken to determine the possible toxic hazard from the use of modern insecticides in the Mississippi Delta. In all studies reported no evidence could be found that pesticides were the direct or indirect cause of any chronic disease or a contributing cause in diseases generally recognized as having other etiologies. A definite problem exists in relation to widespread use of toxic compounds by inadequately trained and sometimes careless people.

WITH THE INTRODUCTION OF MODERN economic poisons of the chlorinated hydrocarbon and organic phosphorus groups, agricultural practices have undergone considerable revision. The widespread use of these toxic compounds obviously suggests the possibility of acute or chronic intoxication of the resident and particularly of the worker population. It is proper that all physicians should be alert to the possible dangers of new pesticides and that a continuing study of the toxicology of these materials should be made by trained specialists. This point of view should not be confused with the opinion expressed in a few highly publicized reports, both in the popular press (*5*) and in medical literature (*1*, *2*, *4*), which have caused considerable alarm.

The Yazoo-Mississippi Delta is an almost exclusively agricultural area specializing in cotton production. Large quantities of various insecticides are used, primarily for control of cotton insects. During the past 3 years, the Mississippi State Board of Health has been requested repeatedly to determine whether the large-scale use of the newer insecticides presents a toxic hazard to the population of the Delta. This board, being unable to initiate such an investigation at the time, relayed the requests to the Technical Development Branch of the Communicable Disease Center. Because such an investigation had not only local but also general interest, a study was begun in June 1952. Operations were centered at the Cleveland, Miss., Field Station, which is located in the heart of the Delta.

The Yazoo-Mississippi Delta is a flood plain lying between the Yazoo and Mississippi Rivers in northwestern Mississippi. It includes all or almost all of eleven counties and parts of seven more, as shown in Figure 1.

The area is farmed intensively and, because of its physical characteristics, lends itself readily to mechanized cultivation. Many large plantations of several hundred to several thousand acres are located there.

The Delta has been described as "one of the most highly specialized cotton-producing areas in the world" (*6*). This is perhaps not quite as true now as formerly, for some other crops are be-

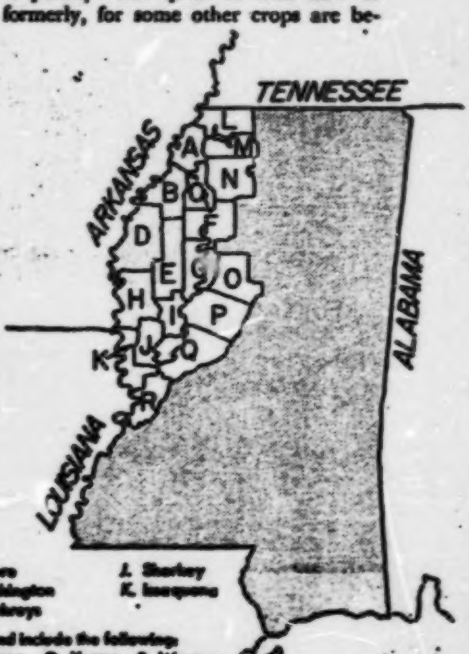

Figure 1. *Map of the State of Mississippi showing the Yazoo-Mississippi Delta*

Counties lying entirely or almost entirely in the Delta are stippled and are as follows:

A. Tunica	D. Bolivar	G. Leflore	J. Sharkey
B. Coahoma	E. Sunflower	H. Washington	K. Issaquena
C. Quitman	F. Tallahatchie	I. Humphreys	

Counties lying partially in the Delta are not shaded and include the following:
L. DeSoto M. Tate N. Panola O. Carroll P. Holmes Q. Yazoo R. Warren

Headwaters of the Yazoo River pass through Arkabutla Reservoir lying between DeSoto and Tate counties; the river flows south to join the Mississippi.

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coming economically important. However, the Delta still accounts for about half of the cotton produced by the state of Mississippi. Roughly, 50 to 60% of the cropland is in cotton, 12 to 16% in soybeans, 5 to 8% in oats, and 5% in hay. The remainder of the land is occupied by miscellaneous crops, pasture, gardens, and idle cropland. Rice is rapidly assuming a position of importance, and livestock production (beef, hogs, and sheep) has become a major enterprise on a few farms (3).

Cotton requires more treatment with insecticides than do the other crops raised in the Delta. Several different compounds are employed for cotton insect control, both as dusts and as sprays, utilizing ground equipment (tractor spray and dust rigs) and air equipment. However, since airplane application of dust and sprays is considered by the local farmers to be less economical and perhaps less effective than ground equipment, it is used only in the latter part of the season when the plants would be damaged by running tractors through them. Few other compounds in the area are aldrin, dieldrin, and toxaphene with the addition of DDT when needed for bollworm control. In addition, benzene hexachloride is used widely in the airplane dusts, frequently in combination with DDT and sulfur in the percentages 3-5-0, 3-10-0, 3-5-40, or 3-10-40 (gamma isomer of BHC-DDT-sulfur). The organic phosphorus insecticides are utilized sparingly, chiefly against infestations of red spiders and aphids which tend to occur in the latter part of July and in August.

Cotton is usually planted beginning the second week in April. Early season insect control is directed against cutworms, thrips, boll weevils, and other minor pests which have survived the winter. Application may be required as soon as the first pair of leaves have spread and may be repeated three or four times at approximately weekly intervals. Mid- and late-season schedules are dependent upon the extent of infestation with the boll weevil and other pests, a 15 to 25% boll infestation demanding three or more treatments at 4- or 5-day intervals. In an average season, approximately 10 applications of insecticide will be required; the range is 6 or 7 to 20 or more applications. Hot dry weather, such as prevailed through the summer of 1952, greatly lessens the amount of poisoning required, because of lessened insect infestation. For example, one large plantation used only about 25,000 pounds of insecticides during the 1952 season as compared to 250,000 pounds during the 1951 season. The poisoning season is usually completed by September 1.

Methods of Investigation

The Mississippi State Board of Health originally requested a study because of recurrent questions from the Delta area regarding the possible effect of the newer pesticides on health. Some of the questions, which are similar to statements published by Biskind (1, 2), were as follows:

1. Does exposure to insecticides bring about a change in intestinal flora leading to gastrointestinal upsets and fungus infections such as thrush?
2. Do insecticides, by their selective action on the central nervous system, cause an increase in cases of insanity?
3. Does insecticide dust suspended in the air cause an increase in hay fever, asthma, and sinusitis?
4. Is there a toxicological hazard from residual DDT on the walls of houses caused by the release of phosgene upon exposure to excessive heat—for example, in winter when heated stoves are in proximity to the walls?
5. Do electrolyte disturbances follow exposure to insecticides? For example, does local gastrointestinal irritation result from exposure and lead to vomiting and thus to dehydration, or does direct stimulation of the respiratory center result from exposure and lead to hyperventilation and thus to alkalosis?
6. Is the increased number of infiltrations noted in films taken by mobile x-ray survey units, and frequently interpreted as virus pneumonitis, actually a result of insecticide exposure?
7. As another stress on the organism, may insecticide exposure contribute to increased incidence of diseases of stress and strain, including neuroses and perhaps even cancer and polio?
8. Through repeated or continuous digestive upsets, does insecticide exposure contribute to malnutrition and deficiency diseases?
9. Do the insecticides cause derangements in enzyme systems and contribute ultimately to chronic diseases such as arteriosclerosis and heart disease?

Because these questions were so broad and so theoretical, it was recognized that obtaining undeniable proof of the absence of a toxicological problem would be nearly, if not quite, impossible, although if such a problem were present to a significant degree, it would not be difficult to obtain proof of the fact. It was felt that the best approach would make use of general morbidity data in several ways, comparing figures when the newer insecticides were in use against a time when they were not in use.

After the general view of medical and agricultural experts in direct contact with the local situation had been obtained, existing statistics were evaluated and morbidity surveys were conducted to study the amount and kind of sickness existing under local plantation conditions. An investigation was made of any reported case of suspected poisoning.

In order to increase the probability of reporting, all physicians were approached through the local medical societies. It was urged that suspected cases of poisoning of whatever nature be reported to the nearest county health officer, or directly to the Cleveland Field Station which was headquarters for the investigation. Other groups, including hospital staffs, county agricultural agents, and civic clubs, were also approached for the same purpose.

Opinion of Local Health Officers and Agricultural Agents

In order to evaluate local feeling concerning the problem of cotton-field insecticide intoxication, several county health officers were first consulted. One reported that the doctors in his county had not complained of the problem to him. Another expressed interest in the possibility of intoxication, but stated that it had not become a problem in his county as judged by his contacts with the physicians. A third stated, in summary, that he had been aware that some laymen and physicians had attributed illness to insecticides, but that he had not found convincing evidence that insecticides play a major role in the causation of illness. Still another stated positively that such a problem did not exist in his county. However, one health officer had received many questions similar to those outlined above and, for this reason, was worried that the problem might be a pressing one. He was anxious that an

Table I. White School Attendance in One Delta County by School Year

School Year	Total Possible Pupil-Days of Attendance	Total Pupil-Days of Absence	Absentee Percentage
A. Before Introduction of Modern Insecticides			
1943–44	713,592	50,041	7.0
1944–45	683,641	45,470	6.7
1945–46	689,927	51,151	7.4
1946–47	705,383	46,202	6.6
Total	2,792,543	192,864	6.91
B. After Introduction of Modern Insecticides			
1947–48	707,584	54,270	7.7
1948–49	760,475	56,776	7.5
1949–50	786,756	57,256	7.3
1950–51	799,280	63,706	8.0
1951–52	801,319	58,528	7.3
Total	3,855,414	290,536	7.53
Grand total	6,647,957	483,400	7.26

investigation be launched to determine the true situation.

As the county agricultural agents are intimately concerned with insecticides and are in close touch with the farmers who use them, an expression of opinion from some of their offices was sought. They felt that the insecticides in current use had proved to be safe, although an occasional case of minor poisoning, usually associated with gross carelessness, had come to their attention.

Review of Existing Statistics

School Attendance Records Total school attendance records for all white children in one Delta county were computed and absentee figures were compared for the 1943–47 period, representing the years before the introduction of the modern agricultural insecticides, and the 1947–52 period, representing the time of widespread use of these compounds (Table I). The absenteeism figures were 6.91 and 7.53%, respectively.

No absolute explanation for the small increase in absence can be offered, although there was an increase in total enrollment during the later period and this increase was derived in part from a group of children who were of school age during the earlier period but did not attend school. When this group did enter school they were a relatively poor risk so far as absenteeism is concerned. Attention is called also to the more general sociological differences between the war and postwar periods, which the figures represent to a large degree. Both rates undoubtedly include absence based on socioeconomic factors as well as illness. A similar set of attendance data for Negro schools of the same county was found to be so incomplete and so inaccurate that it could not be used.

Mortality Reports From the annual statistical summary released by the state of Mississippi, mortality figures were obtained for the state as a whole, and for the following twelve Delta counties: Bolivar, Coahoma, Humphreys, Issaquena, Leflore, Quitman, Sharkey, Sunflower, Tallahatchie, Tate, Tunica, and Washington. Death rates for the Delta and for the state for certain diseases considered germane to this study in view of the previously outlined questions, were compared for 3 years prior to, and 4 years following, the introduction of modern insecticides into this area. These figures are shown in Table II.

Death rates decreased in a similar way for both the Delta area and the state as a whole in tuberculosis, dysentery, pneumonia, and deaths from all causes (crude death rate). The Delta showed a decline in death rate for diarrhea, while the state as a whole showed a slight increase. For cancer, the Delta showed no change, while the state as a whole showed an increase. Only in heart disease was there a pronounced rise for the Delta counties, and this was similar to the rise recorded for the state as a whole. Rates for certain diseases, particularly communicable diseases, show a higher figure for the Delta than for the state both before and after the introduction of modern insecticides. This is due in large part to the predominantly white population in other areas of the state as contrasted to the predominantly Negro population in the Delta with the differences in medical care, economic status, and other factors implicit in this distribution.

Table II. Average Yearly Death Rates per 100,000 Population from Selected Diseases

(For periods 1944–46 and 1947–50 for Delta area and entire state of Mississippi)

	Average Yearly Death Rates per 100,000 Population			
	Delta Area*		Entire State	
Cause of Death	1944–46	1947–50	1944–46	1947–50
Tuberculosis (all forms)	46.3	35.2	40.0	27.7
Dysentery (all forms)	3.9	3.1	2.3	1.8
Diarrhea	10.0	8.0	4.1	5.1
Cancer (including leukemia)	70.1	70.5	78.4	86.2
Heart diseases	141.3	189.4	170.0	213.7
Pneumonia (all forms)	27.4	26.9	34.2	28.0
Crude death rate (all causes)	952.0	921.1	936.7	882.5

* Values given for Delta area represent average for 12 counties.

Hospital Records Records of a hospital located on a large Delta plantation (Plantation A) were reviewed for the period of September 1938 through August 1949. This hospital is operated for the Negro tenant population of the plantation, which averages around 2500 persons.

Records of this hospital furnish a more complete account of illness on the plantation than might be expected, because tenants are hospitalized without cost to themselves and are freely admitted, inasmuch as the company operating the plantation feels it is economically sound to prevent serious illness by means of adequate early care. Furthermore, it is apparent that patients eligible for admission to this hospital are drawn from a population which undergoes a greater exposure to agricultural insecticides than the population from which admissions to most general hospitals are drawn.

September 1938 to August 1946 is used as the pre-modern-insecticide period, and September 1946 to August 1949 as the insecticide-exposure period, since 1947 marked the introduction of significant quantities of modern agricultural insecticides on this plantation (Table III). DDT had been used previously in the area as a residual insecticide applied to dwellings. The amount, however, is insignificant when compared to the bulk used for agricultural purposes, and is not considered in this study.

Table III. Yearly Amount of Older and Modern Types of Insecticides Purchased for Use on Plantation A for 1945–52

Year	Older Types, Pounds	Modern Types, Pounds
1945	185,000	500 (DDT)
1946	200,000	0
1947	160,000	234,000
1948	0	195,000
1949	119,200	408,000
1950	0	369,800
1951	0	441,500 lb. 11,875 gal.
1952	0	449,950 lb. 19,525 gal.

Admission rates for selected diseases are compared in Table IV. The same physician was in charge during the entire period (1938–49) covered by the table. It will be noted that the only disease group showing an increased admission rate in the 1946–49 period is the cardiac group of diseases. This probably reflects the rising trend in cardiac disease shown in all recent studies. All other diagnoses showed a lower rate in the latter period. The total admission rate for September 1951 to August 1952 under a different physician who used different criteria for hospitalization, attempting particularly to bring all maternity cases into the hospital, was 27.2 per hundred population.

Morbidity Surveys

Survey Of Workers This survey was done on Negro male laborers, chiefly tractor drivers, on Plantation A. Brief medical histories were taken and physical examinations were done on 83 individuals who were interviewed afterward by the same medically trained investigator at biweekly intervals from July 9 through September 24, a total of six interviews per man. Some individuals could not be reached every time. At each interview, the laborers were questioned closely and individually about any illness or symptoms occurring since the previous interview and about the type of work done since the last interview. Thus, it was possible at the end of the season to divide the group into an exposure group (occupational contact with insecticides at some time during the season), and a

Table IV. Admission Rates for Selected Diseases at Plantation A Hospital for Negro Tenants

Periods represent eras before and after introduction of modern insecticides

Diagnosis	September 19[illegible]8–August 1946[a]		September 1946–August 1949[b]	
	Average annual admissions for specified diagnosis	Average annual admission rate per 100	Average annual admissions for specified diagnosis	Average annual admission rate per 100
Respiratory diseases	188	6.3	102	4.6
Genitourinary diseases	106	3.6	51	2.3
Gastrointestinal diseases	103	3.3	42	1.9
Cardiac diseases	35	1.2	36	1.6
Rheumatism and arthritis	23	0.8	15	0.7
Nervous disorders	18	0.6	10	0.5
Deficiency diseases	5	0.2	1	..
Metabolic diseases	1	..	1	..
Neoplastic diseases	1	..	3	..
Admissions, all causes	833	28.0	488	22.2

[a] Average annual tenant population 2979. [b] Average annual tenant population 2204.

Table V. Illness Reported in Season-Long Morbidity Survey of 83 Negro Male Farm Laborers on Plantation A

Symptom or Disease	Group with Occupational Exposure		Control Group	
	No. times occurring	Rate[a] per interview week, %	No. times occurring	Rate[b] per interview week, %
Headache	18	4.6	16	6.0
Colds	16	4.1	14	5.2
Backache	15	3.9	10	3.7
Skin complaints	10	2.6	..	..
Gastrointestinal complaints	7	1.8	3	1.1
Rheumatism and arthritis	6	1.6	4	1.5
Cardiac complaints	5	1.3	..	..
Infections	4	1.0	4	1.5
Injuries	3	0.8	1	0.4
Hemorrhoids	3	0.8	2	0.8
Allergy	1	0.3	..	..
Toothache	1	0.3	..	..
Miscellaneous	7	1.8	6	2.2
Total morbidity	96	24.9	60	22.4

[a] Based on 386 interview weeks. [b] Based on 268 interview weeks.

control group (no occupational exposure during the interview season); these groups numbered, respectively, 48 and 35 individuals. Information was obtained for 386 interview weeks on the exposure group and 268 interview weeks on the control group. Total illnesses numbered 96 (24.9 per hundred interview weeks) for the exposure group, and 60 (22.4 per hundred interview weeks) for the control group. This is not a statistically significant difference ($p = 0.4$). These illnesses were of the type expected from any population in a general morbidity survey. Signs and symptoms usually associated with insecticide intoxication were not recognized except as described below. This study is summarized in Table V.

An interesting sidelight observed at the initial examination was the high incidence of elevated blood pressure, usually without associated symptoms. Twenty-seven of the 83 (33%) had pressures above 145 systolic or 90 diastolic. These were found to be distributed as follows: 15 in the exposure group (31% incidence), and 12 in the control group (34% incidence), indicating that the two groups were comparable statistically.

The only diagnosis showing a marked difference in the control and exposure group was the frequency of skin complaints. When the records were reviewed, it was noted that this was accounted for by three individuals who complained repeatedly. One had a chronic fungus infection which had been present since about 1918. The other cases involved irritation by insecticides, and are as follows:

One laborer had a chronically infected ulcerated area on one shin which he irritated by spilling some poison concentrate (probably dieldrin) on it.

A worker spilled a dieldrin concentrate on his left leg and did not wash it off or remove it in any way for several hours. About one week later the skin broke down and blister formations and serious exudation occurred. The skin healed slowly under medical supervision, leaving a depigmented area. The role played by the insecticide and that played by the solvent are uncertain in this case.

Other illnesses which could be related directly to insecticides are as follows:

A tractor driver putting out spray of undetermined type (possibly dieldrin) on a windy day had some blown into his left eye. The eye was treated medically, but remained erythematous and burned for several weeks. Recovery was complete.

A tractor driver putting out both spray and dust of an undetermined composition on a windy day complained of burning in the chest for several days thereafter. Examination of the chest was negative. Recovery was apparently complete.

Survey of General Population

The second type of morbidity survey was carried out at another large Delta plantation (Plantation B). An initial rapid morbidity survey was carried out on the personnel living on this plantation, information being obtained on 639 people. This survey was accomplished during the latter part of July 1952, when the use of insecticides was at a minimum because of a long period of hot, dry weather. The survey data concerned only illnesses for the week prior to the interview in order to hold the memory loss factor to a minimum. Results of this survey are recorded in Table VI.

A similar, follow-up survey was carried out in September after the population had been exposed to atmospheric concentrations of insecticides from airplane dusting of the fields. The experience

Table VI. Results of Morbidity Surveys on Plantation B

Illnesses within Past Week	July surveys[a]		September Surveys[b]	
	Number	%	Number	%
Colds and/or sore throats	61	9.6	20	3.4
Headaches	34	5.3	7	1.2
Gastrointestinal complaints	28	4.4	15	2.5
Arthritis and rheumatism	7	1.1	11	1.9
Cardiovascular disease	15	2.3	10	1.7
Infections, including "chills and fever"	7	1.1	5	0.8
Obstetrical and gynecological complaints	7	1.1	5	0.8
Toothaches	6	0.9	2	0.3
Backache	5	0.8	4	0.7
Nervous conditions	4	0.6	3	0.5
Injuries	4	0.6	4	0.8
Asthma	2	0.3	1	0.2
Skin rash	2	0.3	0	0
Miscellaneous	27	4.2	11	1.9
TOTAL	209	32.6	98	16.7

[a] Based on 639 subjects interviewed.
[b] Based on 592 subjects interviewed (3 dead, 44 others not contacted).

of this season was not typical, however, as a total of only about 25,000 pounds of dusts was used as opposed to approximately 250,000 pounds during 1951. This dust, in 1952, was applied to about one third of the cotton by seven applications during August, and that chiefly in the sparsely populated or unpopulated areas. A small amount of defoliant (calcium cyanamide) had also been applied in September to some of the fields which were scheduled for machine picking. Comparisons of the results of this survey with those of the original survey are indicated in Table VI.

Only 592 (93%) of the original 639 individuals were contacted on the follow-up survey. Three of the original group had died, and attempts to reach 44 others were unsuccessful.

Information on the cause of death of the three individuals who died was scanty:

> A 36-year old Negro housewife (G.R.) and field worker died suddenly and unexpectedly in July. She had expressed no complaints during the initial interview. The doctor who saw her at the time of death expressed the opinion that she had died of a heart attack.
>
> A 70-year-old Negro man (S.O.) died in August after a prolonged illness. At the time of the initial interview he was under medical care for heart and kidney trouble and had ascites.
>
> A 70-year-old Negro man (G.M.) died in August after a prolonged illness characterized by abdominal pain and emaciation. At the time of the initial interview he was under medical care but no diagnosis had been expressed. Malignancy appears to be the most likely explanation.

The total morbidity was approximately half that of the original survey. This decline in the second survey was accounted for mainly by the respiratory group, headache group, gastroenteritis group, and miscellaneous group of diseases. The reduction in headaches was probably due to cooler weather, since many of the original attacks occurred during the middle of the day in persons doing physical labor when environmental temperatures usually exceeded 100° F. The decrease in gastroenteritis is typical of the seasonal incidence of the disease. An explanation for the decline in the other two groups is not so apparent. Arthritic complaints increased slightly, as could be expected from the changing weather (onset of lower temperatures). Finally, a lower rate would have been obtained in the second survey, even if all the subjects who were not available for questioning at that time had, in fact, been sick.

Reports of Poisoning Cases

It was anticipated that a certain number of cases of acute poisoning would be uncovered because of the widespread use of toxic compounds by a group of inadequately trained and sometimes careless people. The limited number of such cases found is rather surprising in view of the publicity given to the request for complete reporting. In addition to the four cases in the Plantation A survey, the following cases were discovered through other sources:

> One case of acute mild aldrin poisoning occurred in a Negro tractor driver who tried to clean clogged spray nozzles by blowing through them. His face, arms, and legs were thoroughly saturated, and he probably inhaled and swallowed small amounts in addition. Shortly thereafter he developed headache, nausea, burning of the skin, and jitteriness. He was treated with phenobarbital and recovered completely within 48 hours.
>
> Two cases of nausea, vomiting, and weakness occurred in a house where window fans were operating while a plane was dusting a field directly behind the house with 1% parathion dust. Onset of symptoms occurred within 30 minutes of the time the plane began putting out dust. One of these individuals appeared completely recovered within 6 hours, but the other continued to complain of weakness for several days.

No other cases which seemed to have any reasonable connection with insecticides were reported.

Summary and Conclusions

A survey of local expert opinion in the Yazoo-Mississippi Delta area showed that local county agricultural agents who were in close touch with farmers and four county health officers who were in close communication with physicians did not consider that there was any evidence in the general population of widespread chronic intoxication by insecticides. A fifth county health officer felt that the problem might be a pressing one and should be investigated. The county agents were aware of isolated instances of minor poisoning.

A comparison of the total school attendance records for all white children in one Delta county for the 1943–47 period, representing the years before the introduction of the modern agricultural insecticides, with those for the 1947–52 period, representing the time of widespread use of these compounds, showed that the absentee figures were slightly higher for the latter period (7.53%) than for the former (6.91%).

Mortality figures for 1944–46 and for 1947–50 were obtained for the entire state of Mississippi, and an average mortality rate was calculated for twelve of the Delta counties for the same periods. Of the seven causes of death studied, only heart disease showed a pronounced rise for the Delta counties during the modern-insecticide era, as compared with the earlier period, and this was similar to the rise recorded for the state as a whole. These mortality figures indicated a general improvement in health conditions in the Mississippi Delta as well as the entire state.

Morbidity records for a Delta plantation hospital showed a lower rate for all diagnoses except cardiac diseases in the 1946–49 period than in the 1938–46 period.

A morbidity survey on 48 exposed and 35 unexposed laborers showed no marked difference between the two groups except in the frequency of skin complaints. Part of these complaints were caused by fungus disease and part by direct, excessive exposure to insecticides.

A morbidity survey on 639 unselected people living on a large plantation was carried out in July when the use of insecticides was still at a minimum, and was repeated in September after the population had been exposed to atmospheric concentrations of insecticides from airplane dusting of the fields. The total morbidity in September was approximately half that of the original survey.

Seven cases of acute insecticide poisoning were found in the Delta area during the summer of 1952.

No one of the studies reported above can be considered absolutely conclusive. There are variables other than insecticide usage which cannot be ruled out as contributing factors in these studies, especially in the comparison of morbidity or mortality rates for two different chronological periods. However, in all the studies reported, no evidence could be found that pesticides were the direct or indirect cause of any chronic disease, nor a contributing cause in diseases generally recognized as having other etiologies.

A definite, though not extensive, problem exists in relationship to the widespread use of toxic compounds by a class of inadequately trained and sometimes careless people. This problem is in the nature of acute, often mild, intoxication due to excessive exposure which is frequently associated with gross carelessness. Improvement in this aspect may be expected on a long-term basis in view of the intensive and extensive educational program being carried on by physicians, county agricultural agents, agents of insecticide-producing companies, agricultural experiment station staffs, and other interested people.

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Dinitroorthocresol Exposure from Apple-Thinning Sprays

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The sodium salt of dinitroorthocresol (DNOC) has been used agriculturally for a variety of purposes. As a fungicide it has been used as a so-called "eradicant" spray to kill spores during the dormant season. It has proved quite effective as a weed killer. It has been used as an insecticide against a variety of pests, including mites, where it serves as an ovicide as well as an adulticide, and locusts. It is finding increasing use in apple orchards as a means of chemically thinning blossoms.

Though the Food and Drug Administration[1] has set a zero tolerance for DNOC as a residue on raw agricultural produce and the U. S. Department of Agriculture[2] has insisted on a poison label on agricultural formulations of it, nevertheless, farmers, fieldmen, and insecticide salesmen in this country have commonly considered it to be one of the safer materials as compared, for instance, with the organic phosphorus insecticides. This reassuring attitude is in sharp contrast to views held in Europe, where there have been a number of deaths among spray operators who were applying this material. The pattern of one or two deaths each year over a period of several years is remarkably similar to the record for organic phosphorus compounds in this country. Six of the eight cases reported by Bidstrup and Payne[3] are particularly interesting, since they involved no accident, gross carelessness, or other massive acute exposure immediately preceding death. Rather, each illness followed at least two weeks of chronic exposure with few, if any, notable incidents. This is in direct contrast with deaths from parathion or tetraethyl pyrophosphate, which have characteristically followed a single massive dose usually resulting from accident or gross carelessness and frequently without significant previous exposure.

The intensive use of DNOC or its sodium salt in local areas in this country over short periods of time has resulted in persistent yellow stains on the hair, hands, and feet of many workers but has apparently produced no more serious consequences. It was therefore felt desirable to study exposure to the sodium salt under conditions in which it is used as a chemical blossom thinner in the Wenatchee Valley of Washington. The object of this study was to determine whether or not a serious health hazard existed under conditions of use in this area.

The material most commonly used for blossom thinning in the Wenatchee Valley is a slurry containing 19% of the sodium salt of dinitroorthocresol, 5% of sodium butyl naphthalenesulfonate, and 2% of sodium chromate, together with small amounts of sodium chloride and sodium sulfate. The standard recommendation is to apply 1 to 2 gal. of this concentrate per acre in 500 to 1200 gal. of water, depending upon the spray equipment used. The timing of the application

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is rather critical, and it is unusual for any one operator to use this material for more than five days or for more than six hours on any one day. The timing of the spray must coincide with peak bloom or fall within a very few days thereof, which means the dates in the Wenatchee Valley are usually within two weeks of May 1. The temperatures of the Wenatchee Valley vary greatly in different locations, and there is a very wide fluctuation at any one station, with a recorded range for April and May of 21 to 96 F. The average daily maximum for April is 59 F, and that for May is 66 F. The corresponding average minima are 37 and 44 F. The humidity is generally very low, though occasional rains are encountered. Since rain within 48 hours of spraying with Na-DNOC does considerable damage to foliage, some effort is made to avoid spraying when rain seems imminent. Rather strong, drying winds are common, but spraying is halted at any time that the wind rises appreciably.

METHODS AND RESULTS

An estimate of dermal exposure was obtained by exposing absorbent pads on forearms, shoulders, thighs, and back and front of the neck of the operators during one or more complete spraying cycles. The procedure has been reported in detail in connection with its use in estimating parathion exposure.[4] The pads were then extracted with water in a Soxhlet apparatus until no color was visible in the pad. The intensity of the color of the extractant was read in a Model B Beckman spectrophotometer at 370 mμ and compared with a standard curve. The results of these analyses, as presented in Table 1, indicate an average dermal contamination of 63.2 mg. per hour of exposure, assuming that the operator wore a shirt with short sleeves and an open collar and that he wore no hat or gloves and assuming further that his clothing gave complete protection of the area covered. Respiratory exposure was estimated by analyzing the filters from MSA-type respirators worn during one or more complete cycles. The average for 74 respirator samples was 0.4 mg. per hour of exposure. Thus, the respiratory exposure appears to be less than 1% of the dermal and should be negligible.

TABLE 1.—*Dermal Exposure to Na-DNOC per Hour of Spraying*
Calculated on Basis of Short Sleeves, No Gloves, and Open Neck

Unclothed Body Part	Location of Pads	Pads Analyzed, No.	Body Area, Sq. Ft.	Pad Recovery, Mg./Sq. Ft./Hr.	Total Calculated Exposure, Mg./Body Part/Hr.
Face	Shoulders	76	0.70	20.3	14.2
Hands	Forearms	76	0.87	20.9	18.2
Forearms	Forearms		1.30	20.9	27.2
Back of neck	Back of neck	74	0.12	16.3	2.0
Front of neck "V" of chest	Front of neck	74	0.16	9.9	1.6
Total		300	3.15	....	63.2

To provide an estimate of the quantity of Na-DNOC actually absorbed by the spray operators, urine samples were collected at various intervals before, during, and after the exposure period and analyzed using the procedure described by Parker.[5] Of 183 urine samples analyzed, only 5 contained as much as 0.5 ppm of the sodium salt (the lower limit of sensitivity of the method). These five samples contained from 0.6 to 1.3 ppm of Na-DNOC, with an average of 0.8. Harvey and associates[6] found detectable amounts of DNOC in the urine of all five volunteers in the first specimen passed after ingestion of a 75 mg. dose of the toxicant. They reported little correlation between DNOC concentration in urine and that in blood. The excretion of DNOC in urine was found by the abovementioned authors to vary between 1 and 2 ppm following a 75 mg. dose.

In an effort to find a more sensitive estimate of DNOC absorption, blood samples were drawn from several of the operators whose exposure was of exceptionally long duration, within 24 hours of their last exposure and again one or two weeks later, but in any event before any subsequent exposure to parathion or other pesticide that might interfere with the analysis. The plasma fraction of these bloods was extracted and analyzed by the same technique used for urine. Blood samples taken from persons who had no known exposure to pesticides were similarly treated and used for blank determinations. The results of these analyses are presented in Table 2. The highest concentration observed was 4.3 ppm.

TABLE 2.—*Concentration of Na-DNOC Found in Plasma of Spray Personnel at Intervals After Last Exposure*

Case No.	Approx. Total Exposure, Hr.	Conc. of Na-DNOC in Plasma, Ppm, Days After Last Exposure: 1	7	11
1	48	...	2.2	...
2	86	2.5	4.3	1.2
3	25	4.0	2.2	<1.0
4	86	1.4	2.5	2.7
5	19	...	1.6	...
6	5	...	2.4	...

COMMENT

Harvey and his associates[8] showed that daily oral doses of 75 mg. of DNOC produced blood levels in human volunteers from 15 to 20 ppm after three to five days. After this range of concentration had been reached, additional doses caused temporary increases of concentration which frequently were associated with symptoms and which, on one occasion, reached a level of 48 ppm. The same authors presented a few cases involving experimental dermal dosage with Na-DNOC, which clearly demonstrated absorption and which appeared consistent with the findings from animal experiments that DNOC is absorbed less readily through the skin than through the alimentary or respiratory tracts.

The average measured dermal exposure reported in this paper is 63.2 mg/hr., and the corresponding value for respiratory exposure is 0.4 mg/hr. It appears premature to speculate on the relation between these dosages and the oral dosages used in the study of volunteers except to state that our observations are consistent with the conclusion that DNOC is absorbed less readily through the skin than through the gastrointestinal tract. Certainly the highest DNOC blood level (4.3 ppm) observed following occupational exposure was lower than 20 ppm which the work of Harvey and his associates suggests is near the critical level. No symptoms were reported in any of the subjects in the Wenatchee Valley.

Bidstrup and Payne state that it is the custom for men spraying cereal crops with DNOC for weed control in Great Britain to work long hours. The stock solution reported to be used in Great Britain contained up to 50% DNOC as compared with a standard 19% Na-DNOC used in the Wenatchee Valley. The dilute spray reported to be commonest in Great Britain contained 0.5% to 0.8% DNOC, though one of the fatal cases used 2% and two of the others used 0.8% to 1.25% material in their spray tanks. In the Wenatchee Valley the recommended dilute spray is 0.02% to 0.04%. Three of the fatal cases reported from Great Britain had applied from 900 to over 1500 lb. of actual DNOC during the season in which their deaths occurred, whereas the largest amount of actual DNOC known to be applied by any one spray operator in the Wenatchee Valley during one season was 50 to 100 lb.

Thus, in contrast to England, where DNOC has proved a hazard, the use of DNOC in the Wenatchee Valley appears to involve shorter working hours, more dilute stock solutions, more dilute spray solutions, and the application of much less of the compound during a season by any one worker. The measured exposure to DNOC of spray

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operators in the Wenatchee Valley and their urinary and blood levels of the compound indicated that these operators are not subject to a significant hazard in connection with the recommended use of DNOC for thinning blossoms.

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TOXICOLOGY PROGRAM
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Health Hazards of Some Dinitro Compounds

Effects Associated with Agricultural Usage in Washington State

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In a recent study of the exposure of orchard workers using the sodium salt of dinitroorthocresol (Na-DNOC) as a blossom-thinning spray for apples, Batchelor, Walker, and Elliott [1] concluded that these workers were not subject to a significant health hazard in connection with the use of this material in the recommended manner. This conclusion regarding hazard of this compound contrasts with the attitude in Europe, where there have been many illnesses and a number of deaths among spray operators who were applying Na-DNOC. The present paper reports studies, carried out from 1956 to 1959, of the health hazards associated with the use of the triethanolamine and isopropanolamine salts of dinitro-*o*-secondary butylphenol (commonly referred to as DNOSBP or dinitro amine) as a weed control spray in western Washington, and Na-DNOC as a blossom-thinning spray on tree fruits in central Washington. The latter study is a supplement to the work carried out by Batchelor et al.[1] during the 1955 season.

The use of DNOSBP for weed control involves use of more concentrated solutions, higher dosages per acre, and more days of spraying per worker per season than does blossom thinning with Na-DNOC. The use of dinitro compounds as weed control sprays in western Washington appears to parallel quite closely the way in which these materials are used in Europe.

The use of DNOSBP for control of weeds in newly planted fields of peas and corn has been a common practice in western Washington.[6,7] A large proportion of these sprays are applied by commercial or contract sprayers. These contract applicators are, in many cases, farmers who not only spray their own acreage but also hire out their services to others and thus have many days of exposure during the season.

DNOSBP is applied both as a preemergence and a postemergence spray. When using ground equipment, the preemergence spray is applied at the rate of 3 to 5 qt. (2.25 to 3.75 lb. of active ingredient) of liquid concentrate DNOSBP in 30 to 50 gal. of water per acre. A postemergence spray of 2 qt. (1.5 lb. active ingredient) in 40 to 60 gal. of water per acre may be applied instead of, or in addition to, the preemergence application. When application is by airplane, the above dosages in only 5 to 10 gal. of water per acre are used.

Most applications are by ground equipment, consisting of wheel tractors or trucks with spray booms attached at the front or rear and with pumps and tanks mounted on the vehicle. The average spray rig has a 40-ft. boom mounted 18 to 20 in. above the

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ground. The boom is equipped with 25 to 35 nozzles positioned so that the spray is directed downward toward the ground. Tank capacity is 350 to 400 gal.

Materials and Methods

Dermal exposure of spray equipment operators to DNOSBP was determined by exposing absorbent alpha cellulose pads and items of cotton clothing on various parts of their bodies. Contamination of hands was measured by rinsing in water in a polyethylene bag. Respiratory exposure was estimated from the contamination of filter pads from conventional spray respirators worn during spraying operations. Short-stemmed glass funnels having stem openings 12 mm. in diameter were taped over the respirator filter pads in order to prevent impingement and to more closely simulate the nasal passages. Other respiratory studies involved use of uncovered respirator pads. All these procedures have been described in detail by Durham and Wolfe.[8]

All pads and exposure garments were extracted with water in a Soxhlet apparatus. Intensity of color of aliquots of these extracts and of the hand washes was determined in a Beckman Model B spectrophotometer* at 370 mμ and compared with a standard curve.

Urine samples were analyzed for DNOSBP content by a modification of the method of Parker.[4]

Sixteen spraymen were studied in the DNOSBP work. A total of 362 dermal pads, 40 respiratory pads, 100 hand washes, 76 cotton garments, and 41 urine samples were analyzed.

The present study of exposure to Na-DNOC was carried out as nearly as possible under the same conditions as the 1955 study by Batchelor et al.[3] Eleven spraymen were studied and a total of 301 dermal and 44 respirator pad samples were analyzed using the same methods described above for DNOSBP.

Results

Studies of Dermal Exposure to DNOSBP.

The results of the dermal exposure studies to DNOSBP are presented in Table 1. Assuming that the operator wore a shirt with short sleeves and open collar, that he wore no hat or protective gloves, and that his clothing gave complete protection of the area covered, the average dermal contamination was found to be 88.7 mg. per hour of exposure. It may be noted that the hands received a disproportionately large share (87%) of the exposed-skin contamination.

It was observed that few workers wore gloves while spraying. However, some men wore gloves during loading operations while measuring the concentrate and pouring it into the spray tank. Some of the spraymen questioned the value of using gloves at any time, since they become contaminated inside. A test was conducted on one sprayman to determine the protective value of wearing gloves during the loading operation only, as compared with not wearing gloves at all. This man wore his own gloves, which were of a rubberized material with knitted cotton cuffs and were stained yellow inside, indicating some residual contamination with DNOSBP. During spray operations involving complete cycles of loading and spraying, the hand contamination for this sprayman was found to be 22.4 mg. per hour when gloves were worn during loading and 91.1 mg. per hour when gloves were not worn at all. (The over-all average value based on 48 samples for all spraymen tested under the latter condition was 77.3 mg. per hour.) This result indicates that wearing gloves during the loading operation markedly decreased exposure to the hands, as compared with situations in which no protection was used.

Considerable variation in hand exposure was noted between careful and careless workers. In one comparative test in which neither man wore gloves, a careless worker was found to have a manual exposure of 95.5 mg. per hour while a more careful sprayman, who followed other recommended precautions, had an exposure of 29.6 mg. per hour.

In the concentrations used for weed control, DNOSBP stains the skin very badly. Although each washing removes part of the stain as evidenced by discoloration of the wash water, some stain, especially on the hands, may persist for weeks. On the morning following a day of exposure to DNOSBP and after the usual bathing and washing of hands, 2.1 and 2.8 mg., re-

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* Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service.

TABLE 1.—*Dermal Exposure of Spraymen to DNOSBP and Na-DNOC*

Body part	Pad Location or Area Rinsed	Body Area (Sq. Ft.)	DNOSBP			Na-DNOC		
			No. Pads or Rinses Analysed	Toxicant Recovery (Mg/Sq. Ft/Hr)	Calculated Exposure (Mg/Body Part/Hr.)	No. Pads Analysed	Toxicant Recovery (Mg/Sq. Ft/Hr.)	Calculated Exposure (Mg/Body Part/Hr.)
Face	Shoulders	0.70	52	1.6	1.1	62	7.0	4.9
Front of neck; "V" of chest	"V" of chest	0.16	33	3.4	0.5	22	2.3	0.4
Back of neck	Back of neck	0.12	22	1.0	0.1	33	5.6	0.7
Forearms	Forearms	1.30	86	7.5	9.7	65	8.5	11.0
Hands	Hands (DNOSBP) Forearms (Na-DNOC)	0.87	48	..*	77.3	68	8.5	7.4
Total	..	3.15	241	..	88.7	180	..	24.4
Less hands	..	2.28	193	..	11.4	190	..	17.0
Thighs	Thighs	3.75	33	31.7	118.9	44	8.1	30.4
Legs	Legs	2.80	30	4.8	13.5	30	3.3	9.2
Above ankles	Above ankles	—	27	1.6	—	17	0.5	—
Total	..	..	56	.	151.1	111	..	56.6

* Bag rinse measurement for hand area.

spectively, were recovered from the hands of 2 spraymen. Three days after his last exposure, 0.2 mg. of DNOSBP was recovered from the hands of one sprayman.

The results of determinations of potential dermal exposure in the thigh, leg, and ankle areas are shown in Table 1. Ten of 38 samples from the thigh area showed spots of concentrated material spilled during loading operations. The average calculated dermal exposure for the thigh area, based on the 10 samples showing visual evidence of contamination, was 57.4 mg. per square foot per hour of exposure, as compared with 17.1 mg. per square foot per hour for the samples showing no evidence of spillage. Eight of the 30 samples from the front of the legs also showed spillage. The average exposure for the 8 contaminated samples was 6.8 mg. per square foot per hour of exposure as compared with 4.2 mg. per square foot per hour for the 22 samples showing no evidence of concentrate spillage. The average DNOSBP content of 37 samples from the ankle area indicated an exposure of 1.5 mg. per square foot per hour. There was no evidence of contamination due to spillage in this area. The total potential dermal exposure for the lower extremities based on these results was 131.1 mg. per hour of exposure. This figure is somewhat higher than that for the usually unclothed body parts (88.7 mg. per hour).

During warm weather, it is common practice for spray operators not to wear undershirts, especially when wearing coveralls. In order to determine how much DNOSBP reaches the skin area through coveralls under such conditions, 3 spraymen wore white cotton tee shirts next to the skin under the coveralls for from 2.5 to 8 hours of spray operations. At the end of the test periods, the tee shirts were cut from the subjects to prevent cross-contamination from their bodies or clothes. The amounts of DNOSBP recovered from the tee shirts were 0.004, 0.06, and 0.90 mg. per hour, respectively.

A similar test was conducted using the foot portion of white cotton socks to determine if DNOSBP penetrated shoes. The socks were worn during spray operations for from 2.5 to 10 hours. The amount of DNOSBP recovered was 0.004, 0.034, and 0.07 mg. per hour of exposure. The highest value (0.07 mg. per hour) was for a sprayman who wore low-cut canvas shoes. The other 2 wore leather work shoes.

Studies of Respiratory Exposure to DNOSBP.—A test was carried out to determine the importance of impingement of spray particles as a factor affecting measurement of respiratory exposure using the absorbent pad procedure. In determining respiratory exposure, using uncovered respirator pads, a study of 16 samples showed an exposure of 0.47 mg. per hour. Concurrently, a group of 10 samples was collected from respirators that were worn loosely over the face and were plugged to prevent air flow. The entire contamination of this group of pads was thus due to impingement. Analysis of these pads indicated a contamination of 0.36 mg. per hour. These results indicate that when inhalation exposure is measured with use of an uncovered respirator pad a large part of the apparent exposure is actually due to impingement. This conclusion is strengthened by the results cited below for Na-DNOC.

The average respiratory exposure to DNOSBP based on analysis of 8 funnel-covered respirator pads was 0.12 mg. per hour of exposure. This value is considered to represent respiratory exposure. It is interesting to note that if one adds the average respiratory exposure value (0.12 mg. per hour), as measured by funnel-covered pads, and the average impingement (0.36 mg. per hour), the resultant sum (0.48 mg. per hour) almost exactly equals the average respiratory exposure measured by uncovered pads (0.47 mg. per hour).

Comparative Exposure for Workers Using Various Types of Spray Equipment.—A comparative study was made of potential dermal exposure during operation of the 3 types of ground spray machines in general use in this area for application of DNOSBP for weed control. These include

Table 2.—*Comparative Potential Dermal Exposure to DNOSBP for Three Types of Spray Machines**

Unclothed Body Part	Location of Pads	Body Area (Sq. Ft.)	Truck-Front Spray Boom			Tractor-Front Spray Boom			Tractor-Rear Spray Boom		
			No. Pads Analyzed	Pad Recovery (Mg/Sq. Ft/Hr)	Exposure (Mg/Hr)	No. Pads Analyzed	Pad Recovery (Mg/Sq. Ft/Hr)	Exposure (Mg/Hr)	No. Pads Analyzed	Pad Recovery (Mg/Sq. Ft/Hr	Exposure (Mg/Hr)
Face	Shoulders	0.70	10	2.18	1.5	14	1.00	0.7	10	0.89	0.6
Front of neck; "V" of chest	"V" of chest	0.16	6	11.83	1.9	7	0.81	0.1	5	1.30	0.2
Back of neck	Back of neck	0.12	5	0.83	0.1	7	1.03	0.1	5	0.92	0.1
Forearms	Forearms	1.30	12	9.34	12.1	14	1.06	1.4	9 †	2.46	3.2
Total	..	2.28	33	..	15.6	42	..	2.3	29	..	4.1

* Hand exposure was determined only during loading operation and was not applicable.
† Excluding one forearm pad that showed visual evidence of contamination during loading.

(1) open-cab truck with spray boom attached forward in line with the front bumper; (2) wheel tractor with front spray boom, and (3) wheel tractor with spray boom mounted behind rear wheels. It appears from the results of this work, as shown in Table 2, that operators of the truck sprayer were subjected to greater exposure than the operators of the tractor sprayers. This difference may be due to the fact that the truck operator sits at a lower level (with respect to the ground) than the operators of the tractor sprayers and for this reason comes into closer contact with the spray mist.

In contrast to yellow staining of skin and clothing noted in workers using ground spray equipment, no staining of exposed skin areas with the exception of the hands was noted on an airplane pilot who was inspected after a 12-hour day spraying DNOSBP. By use of the bag rinse technique, the contamination of the pilot's hands was found to be 2.9 mg. of DNOSBP, representing an exposure of 0.2 mg. per hour. It was noted that the pilot stained his hands while loading the airplane with spray. However, even during this operation his exposure was kept at a minimum due to the method by which the plane was loaded. The pilot merely poured the concentrate into a drum. The mixing with water and transfer into the airplane spray tank took place within a closed system with a minimum of handling. The pilot did not wear gloves. He wore a canister-type respirator while flying.

The flagman working with the above-mentioned pilot had visible staining of the face and neck as well as the hands from spray drift. His hands were found to have a contamination of 10.3 mg. of DNOSBP, representing 0.9 mg. per hour. He wore no respirator.

Urinary Excretion of DNOSBP.—Urine samples from the pilot and from the flagman mentioned above contained 0.36 and 0.86 ppm of DNOSBP, respectively.

Thirty-nine urine samples were collected from 10 ground spray operators at intervals late in their spray season. The DNOSBP content of these samples ranged from 0.02 to 1.04 ppm with a mean of 0.18 ppm. Calculated in another way, the DNOSBP excretion represented by these samples ranged from 5 to 49 with a mean of 22µg. per hour. Many of the values were near the lower limit of sensitivity of the chemical test.

These urinary excretion values appear to be of about the same order of magnitude as those reported by Batchelor et al.[1] for workers using Na-DNOC. Since dermal and respiratory exposure values as well as urine concentrations of DNOSBP were low, no blood samples were analyzed in the present study.

Exposure of Workers to Na-DNOC During Blossom Thinning.—The results of the dermal exposure studies of workers using Na-DNOC are presented in Table 1. The total dermal exposure value for unclothed body parts shown in the table (24.4 mg. per hour) is significantly lower than that reported by Batchelor et al.[1] for the 1955 season (63.2 mg. per hour). This difference applies to each of the individual body parts studied as well as to the total. The data were reviewed to determine the possible effect of variation in spray concentration, type of application (i.e., handgun or air-blast sprayer), wind velocity during sampling, and placement of exposure pads. No explanation for the apparent variation in dermal exposure between the two seasons is available at the present time.

Respiratory exposure studies were carried out, using 22 paired samples in which one respirator pad was covered with a glass funnel to prevent impingement while the other pad was left uncovered. The average potential respiratory exposure, based on the pads protected by the funnel, was 0.03 mg. per hour as compared to 0.48 mg. per hour based on the uncovered pads. The latter figure (0.48 mg. per hour) compares quite closely with the respiratory exposure of 0.4 mg. per hour reported by Batchelor et al.[1]

Comment

The ground spray equipment used in western Washington for applying weed control sprays is apparently about the same as that used in Great Britain. However, one type of British sprayer is described by Bidstrup and Payne as a machine in which the spray is forced through the boom by an air blast produced by a centrifugal fan.[2] The resultant spray may be atomized into finer droplets than are formed with the low pressure machines (operating at about 40 lb. per square inch pressure), which are found in Washington State. On the other hand, the commonly used British sprayer, the reciprocating pump machine described by Bidstrup and Payne,[2] operated on only 27 lb. per square inch pressure and probably did not break up the spray as finely as the machines used in western Washington, thereby producing less drift.

Dosage and spray concentration also appear to be somewhat similar. The two liquid concentrates used in western Washington contain 53% and 57% active ingredients, respectively. According to Bidstrup and Payne,[2] the commercial preparations in Great Britain used during the period of reported illnesses and deaths were in the form of a paste containing up to 50% active ingredient, which was diluted with water immediately before use. This procedure involved mixing and diluting the material in a container before pouring it into the spray tank. In western Washington the liquid concentrates require no premixing and are merely measured before being poured into the spray tank.

For preemergence application in western Washington, the concentration used is 1 lb. of active material to 8.0 to 22.4 gal. of water. For postemergence application, 1 lb. to 26.4 to 39.6 gal. of water is used. The most commonly used concentration is 1 lb. active material per 13.3 gal. of water. Calculating from the data of Bidstrup and Payne,[2] concentrations used in Great Britain were 1 lb. active material per 15.0 to 24.0 gal. of water with an average of 1 lb. per 18.5 gal. These data indicate that the dilute sprays applied in Great Britain for weed control were somewhat less concentrated on the average than those applied in western Washington.

Since poisoning from these compounds is more prevalent during hot weather, a comparison of temperatures between the two areas was of importance. According to Bidstrup and Payne,[2] the maximum temperature recorded in places in Great Britain on the days when the deaths from DNOC poisoning occurred ranged from 66 F to 86 F with a mean of 72 F. The minimum temperatures on those days ranged from 40 F to 50 F with a mean of 46 F. Maximum temperatures in western Washington during May and June, when DNOSBP spray operations are in progress, often range above 70 F and occasionally rise above 80 F. Maximum temperatures for May and June during the 1956-1959 period ranged from 59 F to 94 F with a mean of 76 F. Minimum temperatures for that period ranged from 30 F to 56 F with a mean of 41 F. Spot check temperatures taken in the field at several locations during the spray operations reported in this paper ranged from 61 F to 79 F with a mean of 69 F. The weed control sprays are applied about the same time of year in both locations.

The possibility should also be considered that the difference between European experience and that in this country might be due to a greater awareness of hazard with consequent closer attention to safety precautions during use in the United States.

The compound most commonly used in Britain has been DNOC, while in western Washington DNOSBP is recommended for weed control.

The toxicology of the alkyl-dinitrophenols used in agriculture has been studied by Spencer et al.[6] They reported that when applied dermally to guinea pigs and rabbits DNOSBP was somewhat more toxic than DNOC. However, on the basis of acute oral administration to white rats, DNOC was slightly more toxic than DNOSBP. *On the basis of the data reported by Spencer et al.,*[6] it appears that the dermal LD_{50} to guinea pigs of DNOC is in the range of 300 to 400 mg/kg while that of DNOSBP is around 150 to 300 mg/kg.

It should be noted that the apparently greater potential dermal exposure for DNOSBP (88.7 mg. per hour) as compared with Na-DNOC (24.4 mg. per hour) may have been at least partly a result of the difference between procedures used for determining the contamination of the hands. When exposure of the usually unclothed body parts exclusive of the hands is considered, the values for DNOSBP (11.4 mg. per hour) and Na-DNOC (17.0 mg. per hour) correspond more closely. The potential contamination of the lower extremities and the respiratory exposure were greater for DNOSBP (131.1 and 0.12 mg. per hour, respectively) than for NaDNOC (39.6 and 0.03 mg. per hour, respectively).

Two points of difference in spray operations that may account for these differences in exposure are as follows: 1. Dosage rate: DNOSBP is applied at the average rate of about 5 lb. per acre while Na-DNOC is used at about 1.3 lb. per acre. 2. Method of spray application: Na-DNOC is directed upward into the fruit tree while DNOSBP is directed toward the ground. The downward spray drift from the Na-DNOC application probably accounts for the greater exposure to the upper part of the body with this compound, while the rebound of the DNOSBP from the ground probably accounts for its larger contamination of the lower extremities.

From the formula proposed by Durham and Wolfe,[3] using the exposure figures given above, and assuming the LD_{50} values at the midpoint of the listed ranges, it may be calculated that in an 8-hour work day the potential exposure for a sprayman using Na-DNOC for blossom thinning would be 0.9% of the toxic level, while for a workman using DNOSBP for weed control the exposure would be 4.6% of the toxic level. Thus, on the basis of this calculation, it appears that the latter activity is potentially *considerably more hazardous to the worker* than is the former job. These values can be compared with the figure of 43.2% of the toxic level determined for a sprayman using parathion in an apple orchard.

During the course of this work, it was noted that very few of the spray operators studied wore any sort of effective protective equipment to prevent skin contamination. Most of the men spraying with DNOSBP wore respirators, but only a few who sprayed with Na-DNOC used any respiratory protection. In view of the large contamination of the hands and of the spillage pattern of the concentrate down the front of the body while mixing, it is believed that the wearing of rubber gloves and a rubber apron while mixing either of these compounds would prevent a large proportion of the exposure of the workers during spray operations.

Summary

Studies were carried out to determine the health hazards associated with the use of certain dinitro compounds (DNOSBP and Na-DNOC) as weed control and fruit tree blossom thinning sprays in the State of Washington. Dermal exposure of spray equipment operators was determined by exposing absorbent alpha cellulose pads and items of white cotton clothing on various parts of their bodies for one or more complete cycles of spraying. Contamination of hands was measured by rinsing in water in a polyethylene bag. Respiratory exposure was estimated from contamination of filter pads on respirators worn during spraying operations.

It was calculated that in an 8-hour work day the potential exposure for a sprayman using DNOSBP for weed control would be 4.6% of the toxic level, while for a workman using Na-DNOC for blossom thinning the exposure would be 0.9% of the toxic level.

It was found that the hands receive a large proportion of the dermal exposure when spraying with DNOSBP. There was considerable variation in hand exposure between careful and careless workers even when not wearing gloves. The importance of wearing gloves, especially during the mixing and loading operations when handling the concentrate material, was also demonstrated.

Operators of spray equipment mounted on wheel tractors were found to get less exposure than those operating open-cab truck equipment. Exposure to pilots and flagmen applying DNOSBP for weed control appears to be comparatively low.

Urinary excretion of DNOSBP in subjects involved in ground application of weed control sprays was low, ranging from 0.02 to 1.04 ppm with a mean of 0.18 ppm.

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Original Articles

Oral Toxicity of Mirex in Adult and Suckling Rats

With Notes on the Ultrastructure of Liver Changes

Thomas B. Gaines and Renate D. Kimbrough, MD, Atlanta

The oral toxicity of mirex and its effect on reproduction was studied in rats. The one-dose oral lethal dose for 50% survival of the group (LD_{50}) in females was 365 mg/kg/day; the 90-dose oral LD_{50} was 6 mg/kg/day. The chronicity factor was 60.8. Females fed 25 ppm mirex had fewer offspring born alive, significantly fewer offspring survived to weaning, and many pups developed cataracts. Females fed 5 ppm produced normal litters. Offspring born to mothers fed 25 ppm mirex and nursed by nontreated foster mothers had a low incidence of cataracts and a normal survival rate to weaning. Gas chromatographic analysis of milk and fetuses showed excretion of mirex in the milk and passage through the placental barrier. Livers of male and female rats fed 25 ppm mirex weighed significantly more than controls. Proliferation of smooth endoplasmic reticulum and osmiophilic dense bodies were observed in these livers with the electron microscope.

MIREX (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene) is used to control ants, wireworms, and earwigs, and extensively in the southeastern United States to control fireants. However, little information is available concerning its effect on mammals.

The acute oral lethal dose for 50% survival of the group LD_{50} for mirex in Sherman strain rats was reported to be 740 mg/kg in males and 600 mg/kg in females.[1] Ware and Good[2] found that 5 ppm mirex in the diet of the BALB/c strain of Swiss mice over 120 days resulted in high mortality and low fertility and fecundity rates. Swiss mice of the CFW strain treated similarly suffered a reduction in fecundity but no increase in mortality.

Egg production was not reduced in laying hens fed 300 and 600 ppm mirex in their diet for 16 weeks but hatchability of eggs from hens fed 600 ppm and survival rate of chicks from hens fed 300 ppm[3] decreased.

This paper reports results of studies on the oral toxicity of mirex in rats, including effects on reproduction and survival of offspring.

Methods

Sherman strain rats were caged individually and weighed weekly, unless otherwise specified. The mirex was technical grade with a purity of 98%.

Basic Toxicity Studies.—To determine the one-dose oral LD_{50}, mirex was given as a 5% solution in corn oil at appropriate levels to eight groups of ten adult female rats each by stomach tube as described by Gaines.[4] Survivors were observed for a minimum of 15 days after treatment.

The 90-dose oral LD_{50} and chronicity factor described by Hayes[5] were determined in five groups of ten adult female rats, each fed 50, 100, 150, 200, and 250 ppm mirex in the diet for 90 days. The rats were distributed into groups according to a table of random numbers. Food consumption was measured daily during weeks 1, 2, 7, and 12 to determine mirex intake in milligrams per kilogram per day. After 90 days' exposure, surviving rats were fed nontreated food and observed for two weeks to allow time for death or recovery of sick animals.

The method of Litchfield and Wilcoxon[6] was used to calculate LD_{50} values.

Reproduction Studies.—Rats were bred by pair-mating for seven days. Those fed mirex were maintained on the treated diet during this period. Each day until insemination was veri-

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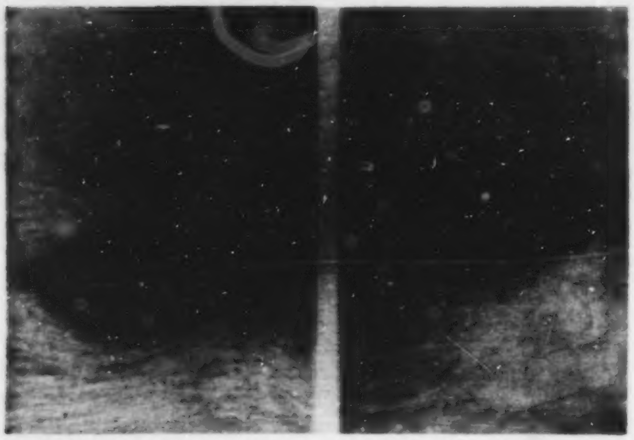


Fig 1.—**Left**, Normal weanling rat. **Right**, Weanling rat of dam exposed to 25 ppm mirex in diet for 102 days at time of breeding. Very pronounced cataract is illustrated.

fied, a vaginal smear from each female was examined microscopically for sperm. By mating mirex-treated rats with "nontreated" rats, both sexes were actually exposed to mirex during the seven days. After seven days, rats were again caged individually. Offspring were examined grossly at birth, during lactation, and at weaning for mortality and development of abnormalities. All litters were weaned when 21 days old.

Twenty female rats, about 6 weeks old, were distributed according to a table of random numbers into two groups of ten each; 20 male rats were similarly divided. One group of males and one group of females were fed 25 ppm mirex in their diet; the other two groups (controls) were fed nontreated diet. Food consumption was measured during the 1st, 6th, and 14th weeks. After 45 days' and 102 days' exposure, the rats fed mirex were mated with nontreated rats of similar strain and age from stock, and the control females were mated with the control males. The females were continued on their respective diets during gestation and lactation. Offspring resulting from the breeding at 102 days of exposure were held after weaning and fed nontreated diet until 90 to 100 days old. They were pair-mated within the respective groups, and their offspring held until weaning and checked for abnormalities.

Since 25 ppm mirex in the diet did not affect male fertility, only females were exposed to mirex in further reproduction studies. Twenty female rats, 6 to 7 weeks old, were distributed into two groups of ten each. One group was fed 5 ppm mirex in the diet and the other the nontreated diet for 102 days. Both groups were bred to nontreated males from stock of the same age and strain. The respective diets were continued during gestation and lactation.

Abnormalities in suckling rats born to treated dams may have been due primarily to excretion of mirex or its metabolites in the milk, or to the sucklings having consumed some treated food after about the age of 2 weeks. Additional studies were made to distinguish between antepartum and postpartum effects of mirex. Forty 6-week-old female rats were divided into four groups of ten rats each, according to a table of random numbers. One group was given 25 ppm mirex in the diet (treated rats); the other three groups nontreated diet. After 49 days' feeding, all females were bred to nontreated male rats of the same age and strain. During the pregnancy and postpartum period, the animals were continued on their respective diets. At birth, offspring of treated dams were transferred to foster mothers fed nontreated diet and their offspring transferred to treated dams. Offspring from the two groups that received nontreated diets (controls) were switched between the two groups, allowing us to observe the influence of the transfer on morbidity and mortality of sucklings.

Mirex in the milk of lactating dams was checked by chemical analysis of stomach contents of two 4-day-old litters of rats from dams treated 78 days and two litters of 4-day-old rats from nontreated dams. Litters were removed from mothers for six hours and then returned to nurse. One hour later, stomachs of the offspring were removed and the contents pooled by litter for analysis.

Four treated dams and four nontreated dams were killed on the 19th day of gestation; four fetuses from each dam were taken for chemical analysis. Stomach contents and fetuses were frozen until analysis for mirex by electron-capture gas chromatography.

Pathology.—Groups of 20 rats (ten males and ten females) were fed 25, 5, 1, and 0 ppm mirex in the diet for 166 days, then killed and the livers studied by light microscopy (in a preliminary study, morphological change was observed only in the liver). Tissue was fixed in buffered 10% formaldehyde solution; routine hematoxylin-eosin stains were used.

Electron microscopic studies were performed on liver tissue from five male and three female rats fed 25 ppm mirex and two males and two females fed nontreated diet (controls) for 166 days. Tissue was fixed in chilled 5% buffered glutaraldehyde for two hours, postfixed for two hours in 1% phosphate buffered osmium tetroxide, dehydrated through changes of alcohol and propylene oxide, and embedded in resin (Maraglas). Sections were cut with a glass knife. The tissue was stained with lead citrate[7] and examined with an electron microscope. The animals were not fasted prior to killing.

Results

Basic Toxicity Studies.—The one-dose and 90-dose LD_{50} values and chronicity factor for adult female rats are included in Table 1. Although the one-dose LD_{50} value of 365 mg/kg is lower than that of 600 mg/kg reported previously for adult female rats,[1] the values, as indicated by the spread of their confidence limits, are not significantly different. The chronicity factor (ratio of the one-dose LD_{50} to the 90-dose LD_{50}) of 60.8 indicates that mirex is highly cumulative. Rats fed the highest dietary level of mirex (250 ppm or 11.6 mg/kg/day) died in 16 to 60 days. Signs of poisoning were diarrhea, weakness, and hyperexcitability.

Reproduction Studies.—Results are shown in Table 2. Reproduction in male rats fed 25 ppm and in females fed 5 ppm and bred to nontreated rats was comparable with that in the controls. Females fed 25 ppm mirex (treated) for 45 days and then bred to nontreated males had significantly smaller litters than controls; the survival rate to weaning was significantly reduced ($P < 0.05$) and 33.3% of the offspring developed cataracts (Fig 1). Compared to controls, the same females bred after 102 days' exposure had fewer litters; the survival rate of offspring was significantly lower ($P < 0.05$) and 46.2% of the offspring developed cataracts. When rats developed cataracts, they were present by 21 days of age. No cataracts were observed in offspring of control rats nor in the parent stock fed mirex.

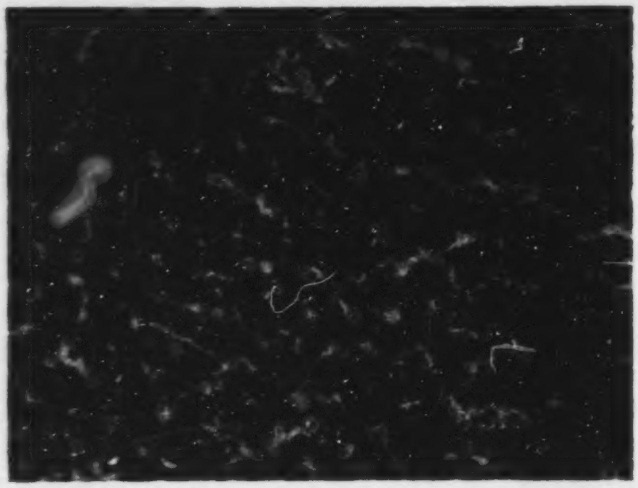
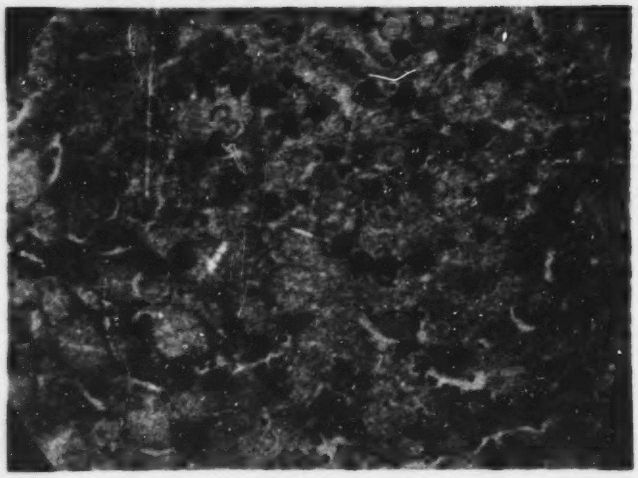


Fig 2.—Normal rat liver (hematoxylin-eosin, reduced from × 300).

Fig 3.—Liver of male rat fed 25 ppm mirex for 166 days showing enlargement of cells, bile pigment, and inclusions (arrows) within cytoplasm (hematoxylin-eosin, reduced from × 300).



Ten male and ten female offspring of females treated for 102 days were fed nontreated diet after weaning and pair-mated at 90 to 100 days of age. At the same time, ten male and ten female offspring of controls were pair-mated. In litter size and survival rate, the F_2 generation (descendants of mirex treated females) were comparable with the control F_2 generation; none developed cataracts.

Table 3 shows the antepartum effect of mirex (litters born to dams fed mirex and transferred at birth to nontreated foster mothers) and its postpartum effect (rats born to nontreated dams and transferred at birth to treated foster mothers). The survival rate to weaning of rats (controls) transferred at birth from dams fed nontreated diet to foster mothers fed nontreated diet was 95.8%; none developed cataracts. The survival rate of rats exposed to mirex in utero only was lower than that for controls (72.7% vs 95.8%), but this was not statistically significant; only 1.6% developed

Table 1.—*Oral Toxicity of Mirex to Adult Rats*

Parameter	1-Dose	90-Dose
No. of rats tested	70	50
Survival time (days)	2-12	16-74
LD_{50}*	365	6.0
19/20 confidence limits*	281-475	4.4-8.2
Lowest dose to kill*	100	6.2
LD_1*	60	1.8
Lowest dose causing clinical signs*	100	6.2
Lowest dose tested*	50	3.1†
Chronicity factor (1-dose LD_{50} ÷ 90-dose LD_{50})	60.8	

* Expressed as milligrams per kilogram for one-dose test or milligrams per kilogram per day for 90-dose test.

† Dietary concentration of 50 ppm.

Fig 4.—Normal rat liver. **SER**, Smooth endoplasmic reticulum; **RER**, rough endoplasmic reticulum; **G**, glycogen; **M**, mitochondrion; **N**, nucleus (lead citrate, × 10,600).

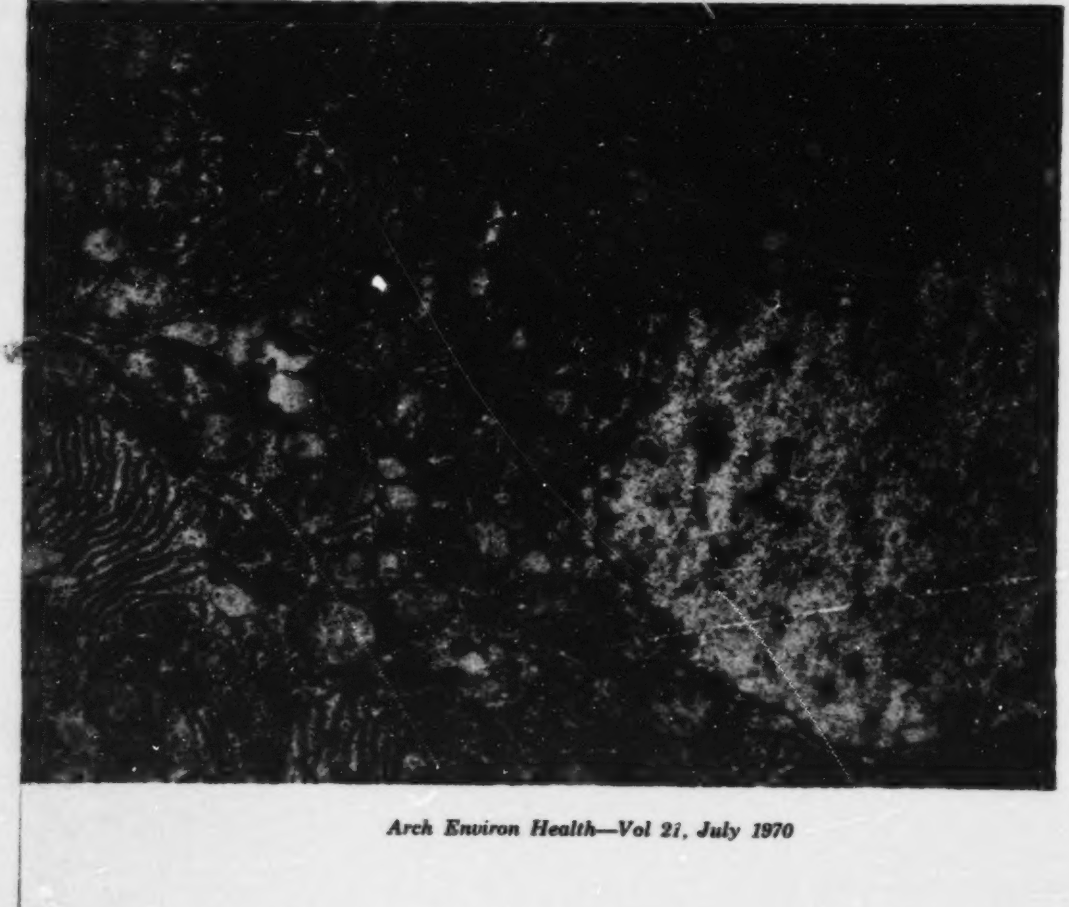


Table 2.—*Survival of Offspring and Incidence of Cataracts in Litters of Rats When One Parent Is Fed Mirex in the Diet*

Sex Poisoned	Dietary Level (ppm)	Dosage Level (mg/kg/Day)	Days on Diet*	No. of Matings	No. of Litters	Average No. per Litter		Survival (%)	Offspring With Cataracts (%)
						Born Alive	Weaned		
None†	0	...	...	10	9	12.0	10.7	89	0
F	25	2.8-1.8	45	10	8	8.5‡	4.5‡	53	31.3
M	25	3.1-1.3	45	10	10	11.4	10.1	89	0
None†	0	...	...	10	9	12.0	11.3	94	0
F	5	0.49-0.31	102	10	9	11.2	10.9	97	0
F	25	2.8-1.8	102	10	6	10.7	6.5‡	61	46.2
M	25	3.1-1.3	102	10	10	12.5	11.5	92	0

* At time of mating and continued on same diet during gestation and lactation.
† Control animals, male and female.
‡ Significantly less than controls ($P < 0.05$).

Table 3.—*Survival of Offspring and Incidence of Cataracts in Litters of Rats Born to Mirex-Treated Dams* and Nontreated Dams†*

Treatment		No. of Litters	Average No. per Litter		Survival (%)	Offspring With Cataracts (%)
Mothers	Foster Mothers		Born Alive	Weaned		
None‡	None‡	14	11.9	11.4	95.8	0
Mirex§	None	8	11.0	8.0	72.7	1.6
None	Mirex§	9	9.9	5.3	53.9‖	37.5

* Transferred at birth to nontreated dams.
† Transferred at birth to treated dams.
‡ Control animals.
§ Five parts per million of mirex in diet for 73 days at the time the litters were transferred.
‖ Significantly less than controls ($P < 0.05$).

Table 4.—*Relative Liver Weights and Morphological Changes in the Liver of Rats Fed Mirex (166 Days on Diet; 10 Animals per Group)*

Dietary Level	Sex	Relative Liver Weight		Remarks
		Fraction of Body Weight (%)	Difference From Controls	
0 (control)	M	2.85	...	LM* Normal liver EM† Livers of 3 rats examined; occ myelin figures and lipid vacuoles
1 ppm (0.09-0.04 mg/kg/day)	M	2.99	...	LM 2/10 rats, slightly enlarged liver cells
5 ppm (0.48-0.21 mg/kg/day)	M	2.77	...	LM 5/10 rats, slightly enlarged liver cells, smooth or vacuolated cytoplasm, occ inclusions
25 ppm (3.1-1.3 mg/kg/day)	M	4.19	$P < 0.001$	LM 10/10 rats, enlarged liver cells, multinucleation, cytoplasm smooth or vacuolated, inclusions; 5/10 rats, bile stasis EM 5 rats examined; increase in smooth endoplasmic reticulum, free ribosomes, lipid vacuoles, and myelin figures; less glycogen, osmiophilic dense bodies
0 (control)	F	3.30	...	LM Normal livers
1 ppm (0.10-0.06 mg/kg/day)	F	3.47	...	LM Normal livers
5 ppm (0.49-0.31 mg/kg/day)	F	3.66	...	LM 3 of 10 rats had enlarged liver cells and vacuolated cytoplasm
25 ppm (2.8-1.8 mg/kg/day)	F	4.24	$P < 0.025$	LM 5 of 10 rats had enlarged liver cells, multinucleation, cytoplasm smooth or vacuolated, inclusions EM Livers of 3 rats examined; increase in smooth endoplasmic reticulum and free ribosomes, myelin figures; osmiophilic dense bodies

* LM, light microscope.
† EM, electron microscope.

cataracts. When rats born to nontreated mothers were exposed postpartum only to mirex-treated mothers until weaned, their survival rate was significantly lower than that for controls (53.9% vs 95.8%, $P < 0.05$); 37.5% developed cataracts.

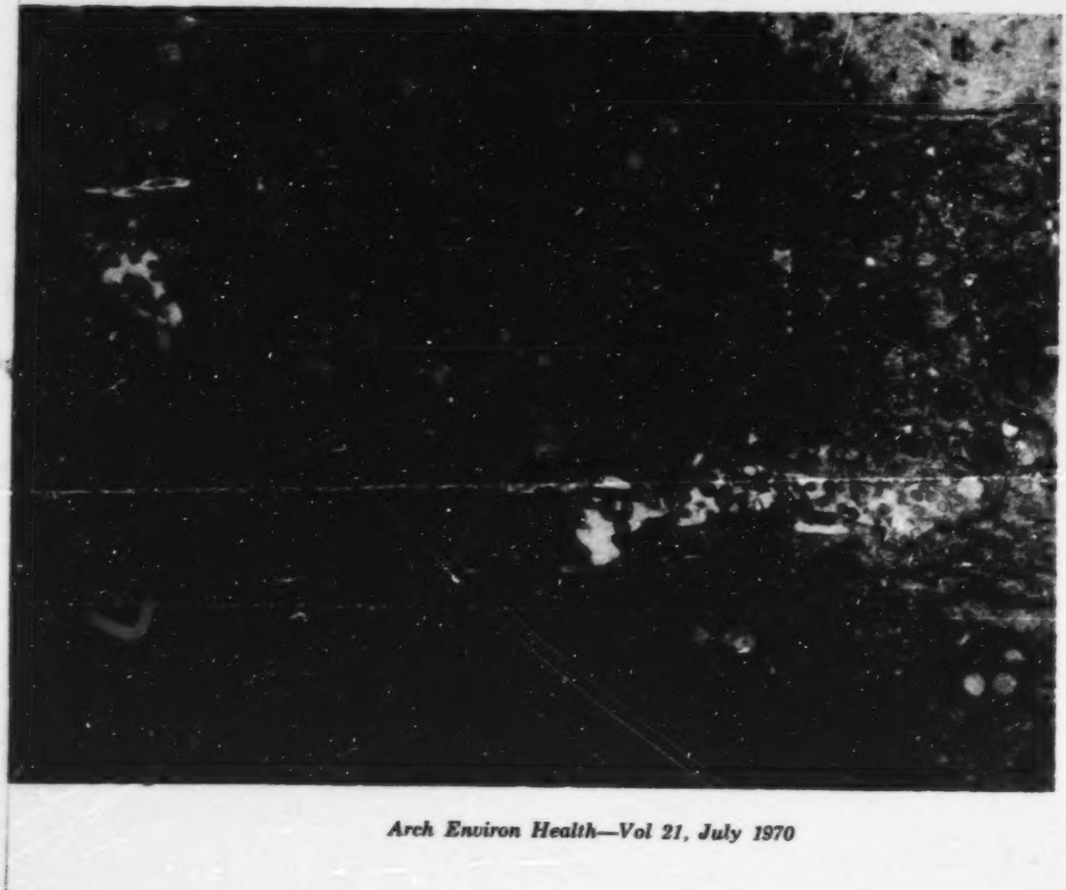
Table 3 indicates that the low survival rate and development of cataracts in offspring of treated dams (Table 2) was caused chiefly by mirex in the mother's milk. The stomach contents of two litters of 4-day-old sucklings born to mothers treated for 78 days were taken just after nursing and analyzed for mirex; the mean value was 11.3 ppm. No mirex was detected in the stomach contents of control sucklings.

Fetuses from four rats treated for 78 days were taken on the 19th day of gestation and analyzed. They contained 0.14 to 0.45 ppm of mirex (mean, 0.23 ppm); none was detected in fetuses from controls. Apparently, mirex passes through the placental barrier in rats.

Pathology.—The weights of brains, hearts, kidneys, lungs, and spleens in rats treated for 166 days did not differ significantly from those of controls. Table 4 shows relative weights of livers and a summary of morphological changes seen with the light and electron microscopes in livers of rats fed 0 (control), 1, 5, and 25 ppm mirex in the diet for 166 days.

Light microscopic changes (Fig 2 and 3) included enlargement of liver cells with either vacuolated or smooth-appearing cytoplasm containing one or more eosinophil-staining inclusions. Livers of three female and five male rats given 25 ppm mirex in

Fig 5.—Liver from male rat fed 25 ppm mirex in diet for 166 days. **M**, Mitochondrion; **G**, glycogen; **SER**, smooth endoplasmic reticulum; **RER**, rough endoplasmic reticulum; **DB**, vacuolated dense bodies probably representing atypical lysosomes; **N**, nucleus; **CB**, cellular border. Note increase in smooth endoplasmic reticulum, decrease in glycogen (lead citrate, × 10,600).



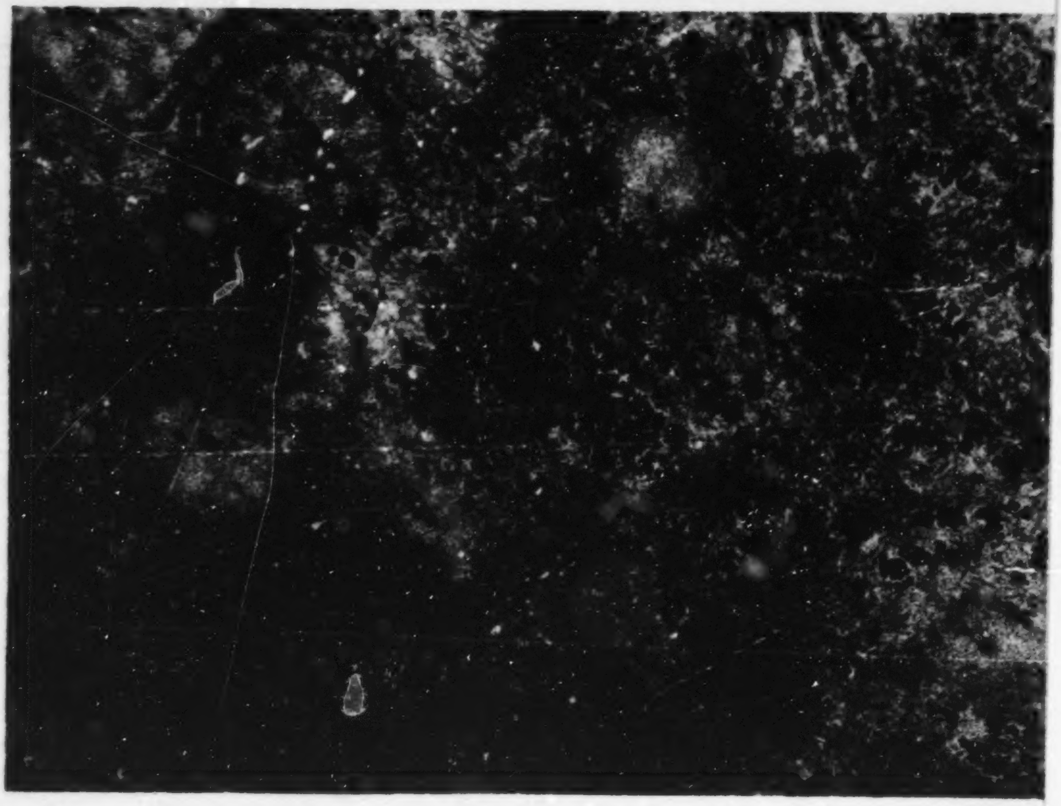
their diets, examined under the electron microscope (Fig 4 to 6), showed an increase of smooth endoplasmic reticulum. Some areas of a number of cells showed an accumulation of free ribosomes. Lipid vacuoles (also seen in control rats) increased; glycogen content in glycogen storage areas of the cells decreased. The cytoplasm of exposed rats exhibited osmiophilic electron-dense bodies, possibly large lysosomes which contained small lipid vacuoles surrounded by varying amounts of dark granular material. Small and large myelin figures were also observed. The bile canaliculi showed no apparent ultrastructural changes.

Comment

Mirex given as an acute oral dose is not very toxic. The 90-dose LD_{50} studies show that it has a very high chronicity factor of 60.8 (ratio of the one-dose LD_{50} to the 90-dose LD_{50}). In contrast, the chronicity factor in female rats is only 5.4 for DDT and 12.8 for dieldrin. These findings indicate that mirex is highly cumulative in effect; like many other chlorinated hydrocarbons, it is probably metabolized or excreted very slowly.

The number of offspring is reduced in treated dams. It is not clear whether this is due to death of the fetus in utero or to a lower fecundity rate. Cataracts develop predominantly in pups in the suckling period. However, when offspring are transferred from mirex-fed mothers to nontreated foster mothers at birth, the incidence of cataracts is very low. Chemical analyses of fetuses of dams exposed prior to birth and of the milk of exposed dams indicates that mirex passes

Fig 6.—Liver from male rat fed 25 ppm mirex in diet for 166 days. M, Mitochondrion; SER, smooth endoplasmic reticulum; G, glycogen; MF, myelin figures (lead citrate stain, × 42,600).



through the placental barrier and is excreted in the milk. Thus, the offspring are exposed to mirex both in utero and after birth, but it is the presence of mirex in the milk after birth that leads to the high incidence of cataracts. If dams are fed 5 ppm mirex for 102 days at the time of breeding and continued on this diet until their offspring are weaned, the pups do not develop cataracts. The number of animals born alive and the survival rate to weaning are also normal.

The effect of mirex on livers is like that of other chlorinated hydrocarbons such as dieldrin and DDT; liver weight increases. Individual hepatocytes are larger than those of controls. Mirex also causes bile stasis, which has not been observed with DDT.

Electron microscopic studies show increased smooth endoplasmic reticulum, characteristic of other chlorinated hydrocarbons. The dense bodies (Fig 5), probably atypical lysosomes, have not been observed in livers of rats fed DDT.[8] According to Ortega,[8] accumulations of lipid surrounded by several concentric layers of membranes are observed in the livers of rats given high doses of DDT but not in mirex-treated rats (also according to unpublished data of one of us [R.D.K.]).

Goldfischer et al[9] observed a numerical increase and more widespread cytoplasmic distribution of lysosomes in livers of rats with ligated bile ducts and rats treated with icterogenin and 17α-ethyl-19-nortestosterone. They found that these lysosomes or dense bodies had vacuoles which did not contain electron-dense material. Similar bodies were observed in human obstructive jaundice.[10]

Electron microscopically, glycogen was less apparent in liver cells of mirex-treated animals than in controls killed at the same time; it is also less apparent in livers of rats treated with phenobarbital sodium[11] but chemical analysis of their liver tissue for glycogen shows no decrease. Glycogen content was not determined chemically in our treated rats; possibly it is not reduced but only becomes invisible with the electron microscope.

Livers of male rats are slightly more sensitive to mirex than those of females. At 1 ppm in the diet (Table 4) all livers of ten female rats were normal microscopically but two of ten livers of male rats still showed slightly enlarged liver cells.

This study demonstrates that mirex is highly cumulative and causes cataracts in offspring of dams fed mirex. Further studies should include its metabolism in animals and its breakdown in soil. Whether the effect on the liver is an adaptive change or should be considered as liver damage is not known at present in terms of an increase in smooth endoplasmic reticulum, formation of atypical lysosomes, and liver enlargement. Bile stasis, on the other hand, has always been considered a sign of liver dysfunction.

Ralph E. Linder and Richard L. Moore assisted with the animal tests. Estelle C. Gray assisted with the statistical analysis. Ralph W. Jennings, Virlyn W. Burse, and Helen T. Mann did the chemical analysis.

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ORGANOCHLORINE PESTICIDE RESIDUES IN HUMAN ADIPOSE TISSUE

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Persistent pesticides, particularly of the organochlorine type, are currently being scrutinized to determine their potential for causing human or environmental harm. Most uses of DDT were cancelled in June of 1972 by EPA order. The production of the insecticides aldrin and dieldrin was suspended for many uses in October of 1974 by ruling of EPA Administrator Russell E. Train. The insecticide mirex, which is used to control the imported fire ant in large areas of southern United States, is currently the subject of a public fact-finding hearing. A large amount of public awareness and interest is focused on these actions.

The presence and distribution of insecticide residues in human tissue have been among the multitude of factors considered in these regulatory decisions. Most of the human residue data were provided by the EPA National Human Monitoring Program. The purpose of this program is to determine the incidence and level of exposure to pesticides experienced by the general population of the United States and to identify changes and trends in these parameters when they occur. This paper reports findings of selected organochlorine residues for survey conducted on human adipose tissue during fiscal years 1970, 1971, and 1972.

METHODS AND MATERIALS

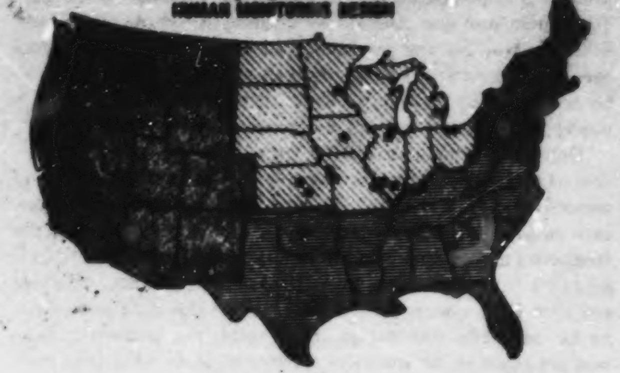
The program was limited to the collection and chemical analysis of samples of adipose tissue that were obtained with the cooperation of pathologists in 32 cities selected in the contiguous 48 states according to a statistical experimental design.

Sampling A proportionate, stratified-random sampling design was followed for selecting cities in which samples were collected. The population strata were the four census regions as provided by the 1960 Census; the sampling units in each census region were cities with populations in excess of 25,000 people. The number of cities sampled in each census region was based on population distribution. The cities that actually served as collecting sites were selected by randomization (Figure 1).

This design provided samples that would be statistically representative of the pesticide residue levels of the general population. For each collection site, an annual sample quota reflective of the national demographic distribution was established. The number in each of these groups was allocated proportionally according to the age, sex, and race distribution in the 1960 Census.

Adipose tissue was collected by cooperating pathologists and medical examiners from postmortem examinations and from specimens excised surgically for therapeutic reasons. Thus, tissues were received from patients having pathological conditions as well as those killed by traumatic injury. Information recorded for each tissue sample analyzed included age, sex, race, and pathological diagnosis. Geographic residence was assumed to be in the general location of the hospital. Since the objective of the program was to reflect the pesticide burden in the general population, samples were not collected from cases of known or suspected pesticide poisonings, from cachectic patients, or from patients institutionalized for extended periods. Further details of the program were presented by Yobs (1971).

SAMPLING SITES FOR FY 70, 71, & 72 HUMAN MONITORING DESIGN



Chemical Analysis All analyses were conducted by contract laboratories using only methodologies specified by the program. These laboratories were equipped with gas-liquid chromatographs with electron capture detectors. All laboratories were required to maintain acceptable performance levels in the interlaboratory quality assurance program, established and moderated by the EPA Pesticides and Toxic Substances Effects Laboratory. This laboratory also served as technical consultants for the analytical portion of the program.

Samples were analyzed for selected chlorinated hydrocarbon insecticides (Table 1), using a modified Mills-Olney-Gaither procedure (Mills *et al.*, 1963; deFaubert *et al.*, 1964). Thin-layer chromatography was used for confirmation of the results of every fifth sample. Results were reported on a wet-weight basis, and the percent lipid extractable material was included for each sample. The exact analytical methods used in this program were published in manual form by Thompson (1972).

TABLE 1
LIST OF CHEMICALS DETECTABLE IN HUMAN ADIPOSE TISSUE

o,p'-DDT
p,p'-DDT
o,p'-DDE
p,p'-DDE
o,p'-DDD
p,p'-DDD
α-BHC
β-BHC
Lindane
δ-BHC

Aldrin
Dieldrin
Heptachlor
Heptachlor Epoxide
Endrin
Mirex
Oxychlordane
Trans Nonachlor
Polychlorinated Biphenyls
Hexachlorobenzene

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Computations and Descriptive Statistics. Residue values presented in this paper were calculated on a percent-lipid basis. This conversion was affected by dividing the whole tissue (wet-weight) value by the proportion of lipid extractable material for each specimen. This method of reporting reduced the inherent variation in residue data attributable to differences in the lipid content of individual adipose tissue specimens.

Data were characterized by calculation of several descriptive statistical parameters: sample size or total number of samples analyzed for the particular residue; frequency or percent positive; and geometric mean estimate. In calculating the frequency and the geometric mean, only reports of quantifiable amounts of pesticides were considered; reports of trace amounts were disregarded. Since these residue data tended not to be normally distributed statistically, the geometric mean was adjudged to be the most reliable measure of central tendency.

RESULTS AND DISCUSSION

The residues selected for presentation in this paper were beta-benzene hexachloride, total DDT equivalent, dieldrin, heptachlor epoxide, oxychlordane, and polychlorinated biphenyls. All, except polychlorinated biphenyls, are representative of exposure to organochlorine insecticides; residues of polychlorinated biphenyls are indicative of exposure to that industrial contaminant.

Beta-Benzene Hexachloride (Table 2). This residue was representative of exposure to the insecticide benzene hexachloride. These data showed that this chemical was widely distributed at low levels in the human population during all three years. Although there seemed to be a trend toward reduction in the quantity of residue, this is not reflected in the frequency, which remained equally high during all three years. There has been a hypothesis that certain animals are capable of converting the various isomers of BHC, including lindane, to the beta configuration for storage (Kamada, 1971). If this is true, then storage of the beta isomer may be representative of exposure to all configurations of BHC.

TABLE 2

NATIONAL SUMMARY OF BETA – BENZENE HEXACHLORIDE RESIDUES IN HUMAN ADIPOSE TISSUE

	FY 1970	FY 1971	FY 1972
Sample Size	1412	1616	1916
Frequency	99.15%	99.32%	99.22%
Geometric Mean	0.37 ppm	0.34 ppm	0.23 ppm

Total DDT Equivalent (Table 3). This conglomerate figure reflected the total burden of DDT and its metabolites found in human adipose tissue. DDT had the distinction of being stored in greater amounts than any other organochlorine pesticides found in adipose tissue during these three survey years. Like other residues discussed in this paper, it was found in almost every tissue analyzed.

Biochemically, DDT is dechlorinated in the human body to DDD, and then either metabolized to the water-soluble and excretable DDA, or excreted directly as DDD. DDE storage is not appreciably derived from ingestion of DDT, but rather by ingestion of DDE previously degraded in the environment from DDT. Since DDE is not effectively eliminated from the body, the result is a gradual increase in the body burden of this chemical. On the other hand, DDT would be broken down and excreted more rapidly than DDE (Morgan and Roan, 1970; Roan *et al.*, 1971).

TABLE 3

NATIONAL SUMMARY OF TOTAL DDT EQUIVALENT[1] RESIDUES IN HUMAN ADIPOSE TISSUE

	FY 1970	FY 1971	FY 1972
Sample Size	1412	1616	1916
Frequency	99.93%	99.75%	99.95%
Geometric Mean	7.87 ppm	7.95 ppm	6.88 ppm
Percentage found as DDE	77.15%	79.71%	80.33%

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[1] Total DDT equivalent (o,p'-DDT + p,p' -DDT) +1.114 (o,p' -DDD + p,p' -DDD + p,p' -DDE + o,p' -DDE)

Dieldrin (Table 4). Dieldrin residues were reflective of exposure to the insecticides aldrin and dieldrin. Aldrin residues are converted to dieldrin quickly after exposure by the human body. Low levels of dieldrin residues were found in almost every tissue collected. These remained appreciably unchanged during the three collection years.

TABLE 4

NATIONAL SUMMARY OF DIELDRIN RESIDUES IN HUMAN ADIPOSE TISSUE

	FY 1970	FY 1971	FY 1972
Sample Size	1412	1616	1916
Frequency	96.46%	99.20%	98.28%
Geometric Mean	0.18 ppm	0.21 ppm	0.18 ppm

Heptachlor Epoxide (Table 5). Residues of this chemical were representative of exposure to two pesticides, heptachlor and chlordane. Since heptachlor is a component of technical chlordane, we believe that these residues were most reflective of exposure to chlordane. This residue was also found at low levels in most of the tissues submitted to the program.

TABLE 5

NATIONAL SUMMARY OF HEPTACHLOR EPOXIDE RESIDUES IN HUMAN ADIPOSE TISSUE

	FY 1970	FY 1971	FY 1972
Sample Size	1412	1616	1916
Frequency	94.76%	96.23%	90.29%
Geometric Mean	0.09 ppm	0.09 ppm	0.08 ppm

Oxychlordane (Table 6). Oxychlordane is the mammalian metabolite of the chloroisomers of the insecticide chlordane. The capability to detect oxychlordane was introduced into our multi-residue approach during the mid-fiscal year 1971 collection year (Biros and Enos, 1973). Thus limited data were available for these three years. Based on these figures, oxychlordane was widely distributed at low levels--but at high frequencies--in humans during this period.

TABLE 6

NATIONAL SUMMARY OF OXYCHLORDANE RESIDUES IN HUMAN ADIPOSE TISSUE

	FY 1971[1]	FY 1972
Sample Size	951	1916
Frequency	93.27%	92.33%
Geometric Mean	0.11 ppm	0.11 ppm

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[1] Methodology for detecting oxychlordane in adipose tissue introduced in mid collection year.

Polychlorinated Biphenyls (Table 7). These chemicals are a group of widely used industrial compounds. They are found in many environmental components and, until an accurate analytical methodology was developed, were mistaken for some configurations of DDT (Yobs, 1972).

Analytical methods permitted only the broad categorization of the residue into one of four groups. Slightly less than 75% of the tissues analyzed showed detectable levels of PCB's. Most of the positives were in the 1-3 ppm concentration range.

The National Human Monitoring Program is a continuing function of the Environmental Protection Agency, and reports of more recent surveys are in preparation. As methodologies become available, the scope of the program will be expanded to include additional residues, metabolites, and other evidences of pesticide exposure (Kutz *et al.*, 1974a and 1974b).

TABLE 7

NATIONAL SUMMARY OF POLYCHLORINATED BIPHENYL RESIDUES IN HUMAN ADIPOSE TISSUE

FY 1972 SURVEY

Concentration Range	Number	Percent
Absent	1067	26.01
<1	632	15.46
1-3	2079	50.68
>3	322	7.85

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Organochlorine Pesticide and Polychlorinated Biphenyl Residues in Biopsied Human Adipose Tissue—Texas 1969-72

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beta

ABSTRACT

Organochlorine pesticide residue levels were determined in 221 samples of human adipose tissue from elective surgery in 1969-72 in the lower Rio Grande Valley of Texas. Standard electron capture—gas-liquid chromatographic methods were used. The total DDT level was 23.18 ppm; the DDE level was 17.37, the highest yet reported for a general population. Dieldrin and β BHC levels were also high: 0.35 and 1.29 ppm, respectively. No decrease in storage levels during the study period was detected. There was no difference due to sex, but Mexican-Americans had significantly higher residues of DDE, p,p'-DDT, and dieldrin than did Anglo-Americans. Polychlorinated biphenyls were detected in 15 samples in 1971 but none were detected in the other 3 years.

Introduction

The presence of organochlorine pesticide residues in humans and other nontarget organisms as a reflection of high lipid solubility and unusual stability has generated much investigation. These studies have attempted to monitor temporal and geographic variations in residues (*1-14*). The effort included epidemiological investigations to define factors modifying residue levels and to detect alterations in morbidity and mortality (*3, 7, 9, 15*). This study reports organochlorine residues in adipose tissue of persons undergoing elective surgery in 1969-72 in the lower Rio Grande Valley of southeast Texas. The study area is a geographically isolated and well-defined semitropical agricultural region. It has been subjected to heavy pesticides usage, predominantly on cotton. Incidental to the investigation of pesticide residues has been the detection of polychlorinated biphenyls (PCB's). These compounds have been detected widely in the environment and in human tissue (*16, 17*). This report also includes data on PCB's in the adipose samples analyzed for organochlorine residues.

Methods and Materials

A total of 302 samples of adipose tissue of approximately 5 g each were obtained from elective surgeries performed at one local hospital in 1969-72. The samples were frozen within 30 minutes of excision and stored in glass containers with foil-lined lids at −18°C until analysis. Demographic data were obtained by chart inspection; 81 samples were eliminated because of ambiguities, inconsistencies, insufficient lipid (<30%), or nonlocal residence. It was not possible to determine a pesticide exposure history but none of the samples came from people with known unusual exposure. Extraction, cleanup, and analyses were performed in compliance with the methods prescribed for Community Pesticide Studies Laboratories (*18*). The extraction and cleanup procedures followed the modified Mills, Onley, and Gaither method (*18, 19*).

The concentrated 6% and 15% diethyl ether in petroleum ether fractions were injected into a Micro-Tek 220 gas chromatograph using a tritium foil electron-capture detector and a 6-ft-by-¼-in. outer-diameter glass column. The column contained one of two combinations: 1.5% OV-17 and 1.95% QF-1 on 100-120 mesh Chromosorb W (HP), or 4% SE-30 and 6% OV-210 on 80-100 mesh Chromosorb W (HP). Operating conditions were: column, 200° C; inlet, 225° C; detector, 205° C; carrier gas, nitrogen; flow rate, 70 ml/min; parallel plate electron capture detector operated at DC voltage to cause ½ full-scale recorder deflection for 50 pg aldrin; attenuation, 10 by 16; operating voltage, 22 v; total theoretical plates, > 3500 for each column.

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Quantitation was by peak height and all residues were calculated on a total extractable lipid basis. When there was any question of peak identity, thin-layer chromatography (TLC) confirmation was obtained. All 6% fractions were scanned by electron-capture—gas-liquid chromatography (EC-GLC) and TLC for PCB's and quantitated visually by TLC using Aroclor 1260 as the reference standard.

Inter- and intralaboratory quality control samples and all standards were supplied by the Primate and Pesticides Effects Laboratory, Perrine, Florida. Quality control samples were stored and analyzed with each batch of study samples and the laboratory was in control for the entire study period.

Recovery studies for all residues except PCB's were consistently above 80% and usually above 90% during the study period; residues are reported in this paper without adjustment. Recovery studies were not performed for PCB's but the method is known to have a precision of ± 50% when Aroclor 1260 is the reference standard (*18*).

The limits of detectability for the residues reported are: 0.01 ppm for heptachlor epoxide, DDE, and dieldrin; 0.02 ppm for β BHC, *o,p'*DDT, TDE, and *p,p'*-DDT; and 0.5 ppm for PCB's. Chemical names appear in the Appendix.

Results

Table 1 presents the mean level of the organochlorine residues found in adipose tissue of residents of the lower Rio Grande Valley of Texas for the study period 1969-72. Table 2 shows the percent occurrence of the same residues. In spite of the greatly decreased use of all these agents since 1968 and the abstinence, due to insect resistance, from DDT usage during the period, the level of these residues did not decrease. There are some apparently random, statistically significant variations but there are no trends.

TABLE 1.—*Mean organochlorine pesticide residues in human adipose tissue from elective surgery—Texas, 1969-72*

		RESIDUE: PPM (MEAN ± STANDARD DEVIATION)[1]							
	No.	DDT-R[2]	DDE	*p,p'*-DDT	TDE	*o,p'*-DDT	DIELDRIN	HE	βBHC
Total	221	23.18±15.36	17.37±10.90	3.48±2.54	.10±.11	.24±.28	.35±.23	.11±.11	1.23±.95
Year									
1969	26	23.04±17.50	16.50±11.86	4.12±2.56	.14±.08	.34±.23	.33±.23	.14±.09	.93±.41
1970	68	21.03±11.15	15.95±8.04	3.07±2.29	.09±.12	.10±.14[5]	.29±.22	.08±.07[4]	1.21±.87
1971	88	22.32±15.79	16.58±11.26	3.44±2.53	.08±.09	.32±.34	.36±.21	.14±.10[5]	1.34±1.21
1972	39	29.29±17.35	22.52±12.10[4]	3.08±2.75	.14±.13	.25±.27	.43±.28	.14±.13	1.20±.66
Sex									
Female	122	23.04±14.72	17.12±10.33	3.62±2.71	.10±.11	.24±.24	.35±.25	.11±.11	1.20±1.10
Male	99	23.22±16.03	17.55±11.56	3.30±2.30	.11±.11	.25±.33	.34±.20	.11±.10	1.26±.72
Ethnic[3]									
M.A.	151	25.75±16.16	19.35±11.73	3.84±2.58	.10±.12	.24±.23	.38±.22	.12±.11	1.23±1.04
Other	70	17.85±10.98[5]	13.31±7.33[5]	2.70±2.28[5]	.08±.08	.24±.38	.27±.18[5]	.11±.10	1.17±.73

[1] When $p > .01$, no notation appears; 4 values $\geqq 10x$ the mean have been eliminated. All residues calculated on a total extractable lipid basis.

[2] DDT-R = DDT + 1.114 (DDE + TDE).

[3] M.A. = Mexican-American (Spanish surname); other = Non-Spanish surname.

[4] $p < .01$.

[5] $p < .001$ (t-test).

TABLE 2.—*Organochlorine pesticide residues in human adipose tissue from elective surgery—Texas, 1969-72*

		PERCENT OCCURRENCE							
	No.	DDT-R[1]	DDE	*p,p'*-DDT	TDE	*o,p'*-DDT	DIELDRIN	HE	βBHC
Total	221	100	100	100	93	81	98	98	99
Year									
1969	26	100	100	100	96	93	89	89	93
1970	68	100	100	99	90	56	99	97	100
1971	88	100	100	100	84	88	100	100	100
1972	39	100	100	100	100	97	100	100	100
Sex									
Female	122	100	100	100	97	84	97	98	99
Male	99	100	100	100	88	77	100	98	100
Ethnic[2]									
M.A.	151	100	100	100	94	88	98	99	100
Other	70	100	100	100	91	64	99	96	99

[1] DDT-R = total DDT + 1.114 (DDE + TDE).

[2] M.A. = Spanish surname; other = Non-Spanish surname.

There are no sex differences in residue occurrence, but people with Spanish surnames have higher residues (total DDT, DDE, *p,p'*-DDT, and dieldrin) than people with non-Spanish surnames ($p<0.001$). Classification by Spanish surname is used as an approximation of Mexican heritage and is certainly only an approximation of ethnic origin.

The mean sample storage intervals were 1.8 months in 1969, 6.7 months in 1970, 11.0 months in 1971, and 6.1 months in 1972. In spite of these rather marked differences there was no correlation between this variable and the residue levels. The mean ages for each year (56 for 1969, 55 for 1970, 54 for 1971, and 52 for 1972) suggest the samples were selected from the same population. There were also no differences in distribution of ethnic group, sex, or type of surgery for any of the study years.

The type of surgery from which our adipose samples were drawn is shown in Table 3. The proportion of lipid in the samples averaged 77.2%. There was no correlation between age and pesticide residues; almost all tissue samples were from adults. Previous reports have shown age correlations in only the youngest groups (*3*).

TABLE 3.—*Classification of elective surgery supplying adipose tissue—Texas, 1969-72*

Inguinal hernia	44%
Umbilical hernia	27%
Incisional hernia	13%
Hernia, other	4%
Lipoma	6%
Other types of surgery	6%
	100%

The detection of PCB's is detailed in Table 4; surprisingly, these residues appeared during one year only: 1971. This group of 15 people, compared to people not having PCB's, did not differ significantly in sex, ethnic background, sample storage time, surgical procedure, or level of pesticide residues; but they were slightly older: 62.5 years vs. 51.5 years ($p<0.01$).

TABLE 4.—*Polychlorinated biphenyls in human adipose tissue from elective surgery—Texas, 1969-72*

	POLYCHLORINATED BIPHENYLS (0 = >0.5 PPM)			
	NO. SAMPLES	% POSITIVE	MEAN, PPM	RANGE, PPM
Total	221	7	0.1	0-9.9
Year				
1969	26	0	0	0
1970	68	0	0	0
1971	88	15	1.7	0.6-9.9
1972	39	0	0	0
Sex (1971 only)				
Male	42	24	2.3	0.6-9.9
Female	46	11	0.9	0.6-1.0
Ethnic Background [1] (1971 only)				
Mex.-Amer.	61	15	0.9	0.6-1.4
Anglo-Amer.	27	22	2.9	0.8-9.9

[1] By surname.

Graphic analysis of the distribution of the seven residues showed them to be skewed somewhat to the right as has been demonstrated previously (*19*). The mean is used as the measure of central tendency for comparison to previous work rather than as the rigorous application of statistical principles.

Discussion

Although use of DDT and most other organochlorine pesticides in the Rio Grande Valley was discontinued one year before this study began, the storage level did not decrease among Rio Grande Valley residents. These pesticides have been replaced by organophosphates. These findings confirm the experience of Morgan and Roan (*9*) but contradict Warnich's findings in Utah (*12*).

Duggan's food monitoring from 1965 to 1970 shows an apparent decrease in residue levels in food (*20, 21*), the major source of pesticide intake. This decrease, combined with a decrease in local use on cotton, should result in a detectable decrease in pesticide levels of human adipose, considering Morgan and Roan's (*22*) demonstration that storage levels are dependent upon continuing intake. The exact changes that may have occurred in pesticide intake via food for our study group are unknown, although it is evident from the data that no dramatic decrease has occurred.

The significant ($p<0.01$) increase in DDE levels between 1971 and 1972 is a perplexing find. There was no corresponding increase, during that year, in TDE or DDT levels as would be expected if there had been an increase in intake via food. In fact, the National Food Monitoring Program has shown that DDT levels are higher than DDE levels for almost all samples examined (*20, 21*).

The conversion of DDT to DDE in vivo has been shown to proceed slowly (*22*) and the stability of DDT levels makes this conversion to DDE unlikely.

A possible explanation for an increase in DDE levels alone could result from the discontinuation of DDT usage in the United States. This would cause an increase in the proportion of DDE, and a subsequent increase in the proportion of DDE found in human adipose. But this theory implies an increased exposure of humans to an environmental source of DDE, which seems unlikely. Further monitoring will be necessary to establish this hypothesis.

The observed differences in DDE, *p,p'*-DDT, total DDT, and dieldrin between Mexican-Americans and Anglo-

Americans probably relate to socioeconomic factors rather than to any genetic differences. This conclusion is based on two studies. Davies (*15*) has attributed increased DDT residues in blacks primarily to their lower socioeconomic status rather than to their race; it has been observed (*23*) that in the Rio Grande Valley a disproportionate percentage of the lower socioeconomic group are Mexican-American.

Since it is unlikely that Mexican-Americans consume a larger amount of animal products, which have been shown to contribute to the bulk of dietary pesticide intake (*20, 21*), the difference in residues could relate to employment, home use of pesticides, or other factors associated with lower socioeconomic status. The lack of information in this area discourages further speculation.

A comparison between the data of this report and the residue levels which have been reported previously reveals that these are the second-highest levels of total DDT (*2*) and the highest levels of DDE yet reported in a general population. The dieldrin and β BHC levels are also high but the heptachlor epoxide levels are not remarkable (*1-15, 21*).

National and international comparisons of specimens in a study of this type are subject to substantial hazards. Some specimens are from biopsies and others are from autopsies. Samples received are handled in various ways, including storage in formaldehyde, freezing in plastic, or freezing in glass. There are significant differences in analytical technique; data are reported as means, medians, and geometric means. Some data are reported on a wet-weight basis and some on extractable-lipid basis. Some authors correct for recovery, and the computation of total DDT sometimes fails to consider a difference in molecular weight of DDE and TDE. At times, the work done in these areas is not even mentioned. However, many of the laboratories generating the data cited above are operating under the same contract and quality control program as is this laboratory; comparisons with such laboratories should be valid.

The etiology of the high residues in our study group is probably intense pesticide usage necessitated by a combination of local agricultural needs and a climate favorable to insects. Exactly how exposure occurs is obscure. Reporting residues on a total extractable lipid basis tends to make our results uniformly about 20 to 25% greater than data determined on a tissue-weight basis but the variability of lipid in adipose samples (31 to 93%) makes this a reasonable correction.

The detection of PCB's in only one year is difficult to explain. The study area is mostly rural with very little industrialization and consequently few sources of PCB's. It is possible that these residues represent immigrants from industrial areas but this has not been demonstrated; as stated earlier, it was not possible to determine a pesticide exposure history of persons in this study. Analysis of all variables identified in the study fails to demonstrate any significant differences between the people with PCB residues and those without, except that the former are slightly older: 62.5 years vs. 51.5 years ($p<0.001$).

Residue levels are in the range of those reported in the Tissue Monitoring Program (*17*) and in the Price-Welch study in Michigan (*16*) but the prevalence is certainly much lower. Without further data it must be assumed that the detection of PCB's in 1971, only, is a random event with no biological significance.

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PESTICIDES IN PEOPLE

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Total Mercury Levels in Selected Human Tissues, Idaho—1973-74 [1,2]

J. Gabica, W. Benson, and M. Loomis

ABSTRACT

Total mercury levels were determined in human tissues taken at autopsy from six hospitals in the three basic geographical areas of Idaho. Of the 242 specimens analyzed, 76 percent contained detectable mercury. Levels were compared with respect to the age, sex, and geographic residence of autopsied individuals. Mean levels detected were 1.04 ppm in kidney tissue, 0.34 ppm in liver, and 0.08 ppm in brain. Mean mercury levels for the three geographical areas were: southeastern Idaho, 0.22 ppm; southwestern Idaho, 0.80 ppm; and northern Idaho, 0.43 ppm. The relatively high means in southwestern Idaho specimens may be related to the preponderance of natural cinnabar deposits in that portion of the State. Mercury levels were higher in women than men for all tissues in both the southwestern and northern areas, but the reverse was true in the southeast. Data were compared with findings of other investigators in an attempt to arrive at background levels of total mercury residues in human tissues.

Introduction

Data on mercury levels in human tissues are limited. Mercury has not been included in most comprehensive studies on trace metals in human tissues. Analytical difficulties may offer a partial explanation for this exclusion (*1, 2*).

Knowledge of background levels of mercury in various human tissues is important because of the possible mutagenic effects or toxic properties of this element and its various compounds. Any use of mercurials as diuretics, antiseptics, cathartics, or pesticides (*3-6*) would presumably contribute to mercury found in various body tissues. Naturally occurring cinnabar ore deposits may be an additional source of exposure in Idaho with its extensive history of mining in which large quantities of metallic mercury were used to extract gold from ore (*7*). Some of this mercury is still present in large quantities in certain locations, especially streambeds throughout the State (*8*).

The present study was intended to be a preliminary screening of a survey of mercury levels in certain tissues of humans and a wide variety of wildlife. Cause of death was noted in each instance but was not compared to mercury concentrations found.

Autopsy and Sampling Procedures

The State was roughly divided into three general areas, northern Idaho, southwestern Idaho, and southeastern Idaho, relating to the locations of the six participating hospitals (Fig. 1). The hospitals, two of which were

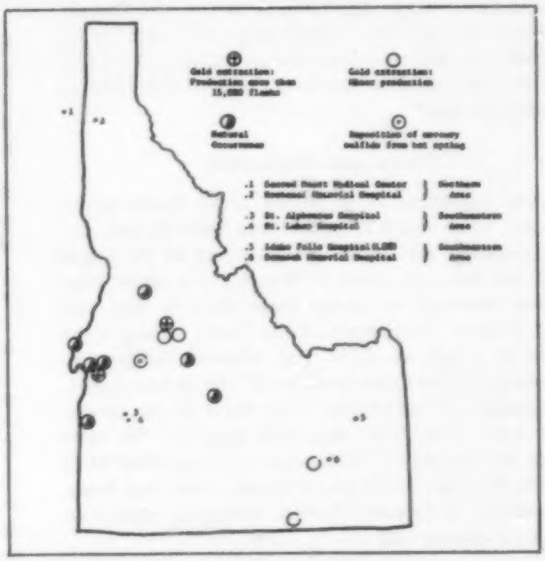
Gold extraction:
Production more than 15,000 flasks
Gold extraction:
Minor production
Natural Occurrence
Deposition of mercury sulfide from hot spring
.1 Sacred Heart Medical Center
.2
Northern Area
.3 St. Alphonsus Hospital
.4 St. Lukes Hospital
Southwestern Area
.5 Idaho Falls Hospital(LDS)
.6 Bannock Memorial Hospital
Southeastern Area
.1
.2
.3
.4
.5
.6

FIGURE 1. *Map of Idaho showing sites of sampling, natural mercury deposits, and gold mining*

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[2] Epidemiologic Studies Program, Department of Health and Welfare, Statehouse, Boise, Idaho 83720.

located in each of these three areas, contributed 242 autopsy samples of kidney, liver, and brain tissues from patients who had died during the previous 12 months from a variety of causes unrelated to mercury poisoning. Because tissues analyzed were from autopsies, they could not be considered normal. However, mean levels drawn from individual cases should be close to normal.

Specimens were placed immediately in formaldehyde at the respective hospitals and sent to the laboratory at once or, in some cases, no more than 2 weeks before analysis. Formalin was analyzed before use and again after autopsy specimens had been stored in the solution. In no instance was mercury or any other background contaminant detected which might invalidate values obtained. Researchers selected homogeneous samples from each organ for analysis, taking care to prevent preanalytical contamination from handling. All glassware used was scrupulously cleaned with nitric acid and rinsed with distilled water.

Tissue specimens were placed on blotting paper and allowed to dry until most of the formalin had been absorbed or evaporated. Subsequently, a 1-5-g sample was ground in a Dual glass tissue grinder; a 1-g wet-weight sample was then removed for analysis. Results are based upon this 1-g sample. The sample was digested according to procedures outlined by the American Association of Analytical Chemists (*9*). Fifty ml distilled water was added to a 50-ml aliquot of the digest and placed in a 200-ml biological oxygen demand (BOD) bottle. Two ml of a 5 percent potassium persulfate solution, 4 ml of a 5 percent $KMnO_4$ solution, 2 ml of a 100-ml water solution containing 12 g NaCl and 12 g hydroxylamine, and 5 ml of a 10 percent $SnCl_2$ solution were added. The mixture was stirred between additions (*8*) and was immediately put under an air vaporizer. Results were recorded on a Coleman 50 analyzer by cold vapor. A standard curve of 0.01-2.00 μg mercury was used.

Results and Discussion

Mercury was found in 76 percent of all tissues tested; the mean value was 0.73 ppm. Mean levels by age, sex, and geographic area of the State for each of the tissues tested are listed in Table 1. Roughly 3.5 times more mercury occurred in kidney tissue than in liver and about 10 times more than in brain tissue. Kidney levels ranged to a high of 15.70 ppm, whereas highest concentrations for liver and brain were 5.80 and 0.94 ppm, respectively. Corresponding mean levels in the current study were 1.04, 0.34, and 0.08 ppm for the same tissues. In comparison, Matsumoto (*10*) reported levels of 6.60, 4.0, and 0.50 ppm in kidney, liver, and brain, respectively, in Japanese fetuses which succumbed to Minamata disease (*10, 11*).

Hospitals with the highest mercury levels in all three organs were located in southwestern Idaho where most of the natural mercury deposits are found (see Fig. 1 in reference *7*). The combined mean level for all tissues was 0.80 ppm in the southwest, whereas the mean level was only 0.22 ppm in the southeast and 0.43 ppm in the north. Nevertheless, it cannot be assumed that these deposits caused the higher levels. High concentrations were not found in this geographical area during a previous study by Benson and Gabica in which 1,000 hair samples from residents throughout the State were analyzed for total mercury (*12*).

Table 2 and the Benson/Gabica study (*12*) show that mercury levels vary according to sex of the subject once they approach or exceed 1 ppm. In general, levels in women were higher than those in men. Women over 65 years of age had more mercury in their tissues than had men in the same age group. The converse was true for people in the 46-55- and 20-45-year age groups although it was less marked in the latter. The higher mercury levels in females of advanced age have not been explained. No available data show evidence of differences in the environmental exposure of males and females. Differences between residue levels in the different sexes cannot be attributed to cosmetics used on skin and hair because the same distribution with respect to sex was found in all organs.

Mean residue concentrations in liver were higher in women than in men from all areas. Women also had higher average levels in the brain and kidney except for kidney tissue in the southeast and brain tissue in the north. Total residues for all three areas were higher among women in all tissues except kidney; in those tissues total values for men were slightly higher (Table 1).

Dal Cortivo et al. (*13*) found that brain, liver, and kidney tissue from autopsy specimens had respective mean mercury levels of 0.05, 0.10, and 0.20 ppm. On the other hand, Kevorkian et al. (*2*) found much higher mean levels: 0.25, 7.70, and 10.36 ppm, respectively.

Hyland et al. (*1*) and Howie et al. (*5*) reported results similar to those of the present study except that Hyland found only 0.65 ppm mercury in kidney samples. Howie's results were corrected from dry to wet weight for comparison by dividing values by five. Takeuchi (*14*) showed that mercury levels in cats averaged 2.00 ppm in the liver, 0.41 ppm in the kidney, and less than 0.10 ppm in the brain. This differs from the findings of the present study and from those of others in which highest concentrations were in kidneys. Takeuchi, however, is in accord with the present study in finding lower mercury levels in human brain tissue than in liver and kidney tissue. This is probably due to the selectivity of the blood/brain barrier, especially with respect to inorganic mercury (*15*). This barrier would not be active in the deposition of mercury into tissue of such organs as liver or kidne[illegible]

TABLE 1. *Distribution of mercury concentrations in tissue from human autopsy samples, Idaho—1973-74*

AREA	TISSUE	SEX	NO. SAMPLES	POSITIVE SAMPLES, %	MEAN, PPM	RANGE, PPM
Southeast	K	M	14	93	1.07	0-3.04
		F	3	67	0.06	0-0.20
		M and F	17	88	0.94	0-3.04
	B	M	13	92	0.02	0-0.06
		F	1	100	0.02	0-0.02
		M and F	14	96	0.02	0-0.06
	L	M	18	78	0.06	0-0.41
		F	8	38	0.15	0-0.25
		M and F	26	58	0.11	0-0.41
	Total	M	45	88	0.39	0-3.04
		F	12	68	0.05	0-0.25
		M and F	57	78	0.32	0-3.04
Southwest	K	M	24	71	1.27	0.02-15.70
		F	22	96	1.90	0.18-12.50
		M and F	46	83	1.57	0.02-15.70
	B	M	9	89	0.11	0-0.16
		F	11	91	0.28	0-0.95
		M and F	20	90	0.19	0-0.95
	L	M	25	100	0.34	0.1-2.11
		F	24	100	0.95	0-5.87
		M and F	49	100	0.64	0-5.87
	Total	M	58	87	0.57	0-15.70
		F	57	95	1.06	0-12.50
		M and F	115	91	0.80	0-15.70
North	K	M	20	75	0.84	0-7.56
		F	11	91	1.14	0-5.78
		M and F	31	83	0.99	0-7.56
	B	M	6	83	0.04	0-0.25
		F	2	50	0.01	0-0.01
		M and F	8	66	0.03	0-0.25
	L	M	18	28	0.06	0-0.49
		F	13	23	0.48	0-5.80
		M and F	31	26	0.27	0-5.80
	Total	M	44	62	0.31	0-7.56
		F	26	55	0.54	0-5.80
		M and F	70	58	0.43	0-7.56
Statewide	K	M	58	80	1.06	0-15.70
		F	36	92	1.03	0-12.50
		M and F	94	86	1.04	0-15.70
	B	M	28	88	0.06	0-0.25
		F	14	80	0.10	0-0.94
		M and F	42	84	0.08	0-0.94
	L	M	61	66	0.15	0-2.11
		F	45	54	0.53	0-5.80
		M and F	106	61	0.34	0-5.80
	Total	M	147	79	0.64	0-15.70
		F	95	73	0.82	0-12.50
		M and F	242	76	0.73	0-15.70

NOTE:
K = kidney
L = liver
B = brain
M = male
F = female

TABLE 2. *Mercury levels in tissue from human autopsy samples, Idaho—1973-74*

AGE RANGE, YEARS	TISSUE	SEX	NO. SAMPLES	RANGE, PPM	MEAN, PPM
0 - 19	B	M	7	0.00-0.73	0.07
		F	3	0.00-0.35	0.14
	L	M	6	0.00-1.11	0.12
		F	7	0.00-5.07	0.95
	K	M	4	0.01-0.64	0.11
		F	5	0.00-1.16	0.37
20 - 45	B	M	3	0.00-0.08	0.05
		F	4	0.06-0.16	0.05
	L	M	8	0.00-0.37	0.19
		F	3	0.00-0.41	0.14
	K	M	8	0.00-3.04	0.77
		F	4	0.05-0.85	0.33
46 - 55	B	M	2	0.00-0.05	0.02
		F	1	0.00-0.04	0.06
	L	M	8	0.00-2.40	0.33
		F	9	0.00-1.06	0.25
	K	M	11	0.01-7.36	2.95
		F	7	0.08-4.40	0.95
56 - 65	B	M	7	0.00-0.16	0.02
		F	2	0.08-0.14	0.08
	L	M	12	0.00-0.31	0.06
		F	8	0.00-2.26	0.63
	K	M	12	0.00-7.56	0.58
		F	7	0.00-0.91	0.50
66 - 75	B	M	6	0.00-0.01	0.00
		F	2	0.00-0.26	0.56
	L	M	16	0.02-0.09	0.11
		F	9	0.00-0.31	0.75
	K	M	15	0.00-0.66	0.26
		F	7	0.04-9.40	5.37
76 - 86	B	M	1	0.00-0.02	0.02
		F	1	0.00-0.33	0.33
	L	M	8	0.00-0.90	0.11
		F	8	0.00-5.80	1.00
	K	M	8	0.02-1.55	0.48
		F	6	0.09-12.50	0.74

NOTE: B = brain
L = liver
K = kidney
M = male
F = female

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Pesticides

Lectures held at the
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Scientific Chairman: Pekka Koivistoinen

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effect [22]. Therefore, the complex effect of exposure to pesticides of different groups should be studied. We compared the effects after simultaneous daily administration of the substance by different routes at doses forming in sum the ED_{50} value (or certain part of LD_{50}) and after isolated oral administration or skin application of ED_{50}.

Changes of cholinesterase activities in serum, erythrocytes, brain, liver, and kidneys (determination by the method of Hestrin [23]) may be indicative of effects of Rogor.

The data on the change of cholinesterase activity obtained after four-months study are presented in Fig. 2. The data show that the cholinesterase inhibition after the simultaneous oral administration and skin application of Rogor is more pronounced than after isolated oral administration or skin application.

A quantitative evaluation of the combined and complex effects may be given on the basis of criteria such as the inhibition-rate of enzyme activity by 50%. The proposed method permits to evaluate quantitatively the combined and complex effects of two or some harmful agents.

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The Views of the United States of America on the Codex Tolerances

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The United States as a member of the Codex Alimentarius Commission has been involved in the work of the Codex Committee on Pesticide Residues (CCPR) since the formation of this Committee. However, up to this point, no national policy has been established concerning what constitutes an acceptance of a Codex international tolerance.

The United States commitment to the Codex pesticide work depends, in large part, on what is meant by *acceptance* of a Codex tolerance. At present, or at least until recently, a Codex general standard may be accepted by a country in accordance with its established legal and administrative procedures in respect to the distribution of products to which the general standard applies, whether imported or home-produced, within its territorial jurisdiction in the following ways:

Full Acceptance:

Full acceptance of a general standard means that the country concerned will ensure, within its territorial jurisdiction, that a product to which the general standard applies will comply with all the relevant requirements of the general standard except as otherwise provided in a Codex commodity standard. It also means that the distribution of any sound product conforming with the standard will not be hindered by any legal or administrative provisions in the country which relate to the health of the consumer or to other food standard matters and which are covered by the requirements of the general standard.

Target Acceptance:

Target acceptance means that the country concerned indicates its intention to accept the general standard after a stated number of years.

Acceptance with Minor Deviations:

Acceptance with minor deviations means that the country concerned gives full acceptance as defined previously to the general standard with the exception of minor deviations which are recongnized as such by the Codex Alimentarius Commission. The country concerned will include in its declaration of acceptance, a statement of such deviations, the reasons for them, and also indicate whether it expects to be able to give full acceptance to the general standard and, if so, when.

The Commission has construed *full acceptance* to mean that countries are obliged to apply the Codex tolerances both to imported foods and to domestically produced foods. This interpretation eliminates the possibility of any "two tolerance" system, i.e., the applying of one tolerance to imports and another tolerance to domestically produced food.

The *target acceptance* is designed to accommodate countries which have not, as yet, developed national tolerance-setting procedures.

Acceptance with minor deviations is subject to many different interpretations and a great amount of confusion exists in this type of acceptance. Classifying deviations as minor or major is a subjective, cumbersome procedure that does not lend itself to uniformity. It serves no useful purpose.

Many countries, including the U.S.A., have expressed dissatisfaction with the definitions of acceptance as applied to pesticides. As a result of the questions raised concerning the Codex procedures on acceptance, a review of this matter has been made by the Codex Committee on General Principles. This Committee, at its March 1974 meeting, recommended that a separate acceptance procedure be established for pesticides.

The *recommendations* (recently ratified in Paris) include acceptance in three forms: full acceptance, limited acceptance, and target acceptance. The recommendations are as follows:

A Codex maximum limit for pesticide residues (Codex tolerance or Practical Residue Limit) may be accepted by a country in accordance with its established legal and administrative procedures in respect to the distribution within its territorial jurisdiction of

a) home-produced and imported food or

b) imported food only, to which the Codex maximum limit applied, in the ways set forth below.

In addition, where a Codex Maximum Limit for Pesticide Residues applied to a group of foods not individually named, a country accepting such Codex Maximum Limit in respect of other than the group of foods, shall specify the foods in respect of which the Codex Maximum Limit is accepted.

Full Acceptance. Full acceptance of a Codex Maximum Limit for Pesticide Residues means that the country concerned will ensure, within its territorial jurisdiction, that a food, whether home-produced or imported, to which the Codex Maximum Limit applies, will comply with that limit. It also means that the distribution of a food conforming with the Codex Maximum Limit will not be hindered by any legal or administrative provisions in the country concerned which relate to matters covered by the Codex Maximum Limit for Pesticide Residues.

Limited Acceptance. Limited acceptance of a Codex Maximum Limit for Pesticide Residues means that the country concerned undertakes not to hinder the importation of a food which complies with the Codex Maximum Limit for Pesticide Residues on that food by any legal or administrative provisions in the country concerned which relate to matters covered by the Codex Maximum Limit for Pesticide Residues; it being understood that in so undertaking the country concerned does not impose by the Codex maximum limit a more stringent maximum limit that is, lower limit, than is applied domestically.

Target Acceptance. Target acceptance means that the country concerned indicates its intention to give Full Acceptance or Limited Acceptance to the Codex Maximum Limit for a pesticide residue after a stated number of years.

A country which considers that it cannot accept the Codex Maximum Limit for Pesticide Residues in any of the ways mentioned above should indicate:

a) in what ways its present or proposed requirements differ from the Codex Maximum Limit for a pesticide residue, and if possible, the reasons for these differences;

b) whether products conforming to the Codex Maximum Limit may be distributed freely, or may be distributed under certain specified conditions, within its territorial jurisdiction insofar as matters covered by the Codex Maximum Limit are concerned.

Briefly, *full acceptance* means that a country will apply the Codex tolerance to both imported and domestic food. Under the *limited acceptance* definition, a country could apply a Codex tolerance to imports only, except that a country could not apply a more stringent tolerance on imports. The new type of acceptance is intended to enable a country to recognize "good agricultural practices" in another country. In the United States, there are two important areas where "limited acceptance" might be considered:

a) Where the Codex tolerance relates to a use of a pesticide which is not a registered use in the United States and, therefore, has had no tolerance established;

b) where the Codex tolerance is higher than the U.S. tolerance and where, for one reason or another, we cannot adjust the tolerance upward.

This, or course, raises the questions again regarding any "two tolerance system". While the U.S.A. is not planning to adopt a "two tolerance" system at this time, we believe that the

General Principles should be flexible enough to allow for this type of distinction between imported and domestic foods, should we decide in the future that circumstances in individual cases warrant different tolerances.

The "limited acceptance" proposal raises many questions of a policy, legal, enforcement, and political nature, questions that may be difficult to answer. But the proposal does give flexibility in the setting of tolerances by an Agency.

In the United States, there are three separate agencies that are vitally concerned with the Codex acceptance procedures: the Food and Drug Administration, the Department of Agriculture, and the Environmental Protection Agency. The Environmental Protection Agency has the responsibility for establishing national pesticide tolerances and the Food and Drug Administration and the Department of Agriculture have responsibilities in the enforcement of these tolerances. The Department of Agriculture also is concerned in that it has responsibilities for foreign trade in agricultural commodities. The Environmental Protection Agency of the United States is relatively new. Various responsibilities held by other Federal Agencies were transferred to the Environmental Protection Agency when it was formed in December, 1970. Various organizational elements in the Agency are now concerned with noise, radiation, toxic substances, solid wastes, air and water quality, and pesticides. So, the Environmental Protection Agency is relatively new, relieving the Department of Agriculture from most of its responsibilities in pesticides, primarily in the areas of registration, experimental permits, tolerances, and enforcement of regulations. The Food and Drug Administration is concerned with pesticides in/on food and feed as well as drugs and other products used on living animals and man. The Department of Agriculture is concerned about meat and poultry entering the U.S.A. as well as agricultural practices which involve pesticides.

In recent months, there has been considerable cooperation between these agencies in establishing procedures for carrying out the work required by Codex. At this point in time, however, no firm policy has been established jointly by the three agencies although several recommendations are being considered. At the Codex meeting, Delegate E.L. Peterson, ARS, USDA has provided a general statement relative to the U.S. position on the proposed changes in the general principles and the U.S. policy on review of Codex proposals.

In brief, the United States concurs in the proposed changes in the acceptance procedures for recommended Codex maximum limits for pesticide residues. We believe that the proposed changes will allow all countries to have the greatest amount of flexibility in giving recognition to, and acceptance of, Codex maximum limits for pesticide residues. Since tolerances for residues of pesticide chemicals in or on raw agricultural commodities may be established only in accordance with the procedures set forth in our national statutes, the acceptance of a proposed Codex tolerance is contingent on the establishment of an equivalent tolerance under U.S. law.

The U.S.A. fully supports the principles of the Codex Alimentarius Commission as they relate to the establishment of international tolerances. Therefore, we will strive to give "full acceptance" to as many as possible of the proposed tolerances recommended by the Commission for acceptance.

It is the policy of the U.S.A. that where Codex-recommended tolerances differ from established U.S. tolerances, we will review each proposal from the standpoint of determining whether changes can be made in the U.S. tolerance regulations. In all cases where possible, action will be initiated under U.S. statutes to make the U.S. tolerance regulations consistent with the Codex proposals. Where the U.S. cannot accepts a Codex proposal for reasons of good agricultural practice or for human health reasons, the reasons for our non-acceptance, and the data upon which our decision is based, will be fully set forth to the Commission.

We know that there are instances where the proposed Codex tolerance is higher than the U.S. tolerance. Fortunately, there are relatively few instances where this is the case; in some, the differences are relatively small, probably analytically or toxicologically insignificant.

Scientific data will support any decision to raise the U.S. tolerance to conform to the higher Codex level. In our review of the proposed Codex tolerances, maximum weight will be given to the determinations by the FAO/WHO Joint Meeting. Within the framework of current law, we will asume the responsibility of making additional information available to these Joint Meetings when we believe that all relevant information was not presented.

The U.S. also supports the principle which would allow a country in accepting a Codex tolerance to apply such tolerance to imports only, while prohibiting or restricting the use of the pesticide domestically. It is important to recognize that the establishment of a tolerance for a pesticide chemical and the registration of the pesticide for use in the U.S.A. are two related, but separate actions.

If the views expressed in this brief paper are adequately followed by action, there will be considerable activity at the interagency level in the United States. We recognize the importance of the Codex Alimentarius Commission and I, personally, belive that we will commit resources – manpower, time, funds – to aid in the solution of the international problems facing the Commission.

The Effect of Chlorfenvinphos Administered in Diets with Different Concentrations of Protein on Rats

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It has repeatedly been reported in recent years that the variation of dietary factors may influence – enhance or reduce – the toxicity and metabolism of various foreign compounds reaching the organism, including pesticides [2, 13]. The role of dietary protein in drug and pesticide metabolism and toxicity is well documented. In experiments on weanling male rats, Boyd [3, 4] observed that diet with protein deficiency (3.5% of casein) increased the acute toxicity of many pesticides administered orally, e.g. the toxicity of DDT was raised 3 times, that of Endosulfan 4 times, that of Carbaryl 6 times, that of Parathion 8 times, and that of Captan 26 times. It was postulated that protein deficiency, which causes degenerative changes and inhibition of growth in the liver, could be expected to limit the production of hepatic detoxifying enzymes.

In contrast to the findings of Boyd [3, 4], Weatherholtz et al. [18] demonstrated that male weanlings rats receiving a 5% casein diet were much less susceptible to Heptachlor than were their pair-fed mates given 20% or 40% casein diets. In this case, the low protein diet impaired the conversion of Heptachlor to the more toxic metabolite Heptachlor epoxide. Casterline and Williams [6] observed that the induction of serum aliesterase activity by Chlordane was less in the rats fed either 8% or 3% casein diets than that in the rats fed 19%, possibly because of an increase in the storage of the pesticide in the fat deposits in the rats on low protein diets. Thus, the problem is not simple and according to McLean and McLean [13]: "There is no clear experimental evidence that the cell's general ability to withstand change and trauma is altered by malnutrition".

It was the purpose of the present study to compare the effect of Chlorfenvinphos on rats kept on diets with 4.5% or 26% of protein.

Materials and methods

The experiments were carried out on 6-week-old female and male Wistar rats of the Institute of Food and Human Nutrition colony. For a period of 30 days, the rats received diets containing 4.5% or 26% of casein with the addition of zero, 5, 100, and 1000 ppm of Chlorfenvinphos. Each group consisted of 50 animals, that is 25 females and 25 males and each variant of experiment was repeated twice. Chlorfenvinphos Ciba obtained from the Institute of Organic Industry in Warsaw was used for contamination of food; the sum of *cis* and *trans* isomers in it calculated with an accuracy of ± 2% amounted to 91.4%.

Diets containing 4.5% or 26% of casein were prepared according to Hegsted and Chang [11] and Boyd [3, 4] with the following modifications: sunflower oil was given in place of hydrogenated cottonseed oil, and wheat starch replaced corn starch. All diets and drinking water were given ad libitum. The amount of food eaten and the body weight gain were determined every day.

After 10 and 30 days, the rats were killed by decapitation without anaesthesia; biological material was taken immediately for enzymatic, histochemical, and histological investigations. The activities of the following enzymes were measured: acetylcholinesterase, AChE (Ellman et al. [8]) and glucosephosphate isomerase, PHI (Bodansky [1]) in the 700 g supernatant from

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HEXANE EXTRACTABLE CHLORINATED INSECTICIDES IN HUMAN BLOOD

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It is generally agreed, on the basis of pharmacological evidence, that the most important site of action of chlorinated insecticides is the central nervous system. This was confirmed in connection with DDT by the fact that the severity of clinical signs of poisoning is directly proportional to the concentration of the unmetabolized compound in the brain (1) and by the fact that the concentration in the brain which leads to death is of the same order of magnitude no matter whether animals are killed by a single large dose or by many small doses (2). Much valuable information may be obtained from analysis of chlorinated hydrocarbon insecticides in the body fat of people in the general population or in any other group whose manner of exposure to the compounds is known. However, in cases in which the exposure is not known, fat is not a satisfactory tissue for analysis, and it is just such cases that are most likely to involve poisoning as a differential diagnosis. The concentration of DDT in the fat, unlike that in the brain, bears no necessary relation to the presence or degree of poisoning (2). Furthermore, samples of fat may be difficult to obtain from some people one would like to study. Samples of human brain are available from some routine autopsy material and may be available in rare cases in which poisoning by a pesticide is suspected as the cause of death, but brain samples are completely unavailable for most diagnostic study or for meaningful monitoring.

Unlike fat or especially brain, blood can be obtained conveniently from almost anyone. There is some evidence that the concentration of DDT in blood tends to correspond to that in the brain and to show far less relation to the concentration in the fat (3). It has also been pointed out that the concentration of a drug at receptor sites is related to the concentration of unbound drug in the plasma (4). Because of its availability and probable diagnostic value, blood would seem to be a good tissue in which to measure

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the concentration of chlorinated hydrocarbon insecticides, and therefore it was studied in this connection.

Several methods have been developed in recent years for the determination of chlorinated insecticides in human blood utilizing gas chromatography and highly sensitive detectors (5, 6, 7). The method described by Robinson (5) utilized alkaline saponification as a clean-up procedure and therefore was unsuitable for the determination of insecticides that are unstable to alkali. The method of Schmit et al. (6) for the determination of DDE, o,p'-DDD, and p,p'-DDT was unsatisfactory in that interfering materials produced multiple large peaks in the early part of the chromatograms where the peaks for several chlorinated insecticides or related materials would also appear. The method described by Kadis and Jonasson (7) is not only time consuming but lacks the sensitivity required for the analysis of these materials in blood of the general population. A rapid, simple, and sensitive procedure for the determination of chlorinated insecticides and related materials in blood of the general population was not available. Since such a method is essential if analysis of blood is to be used for the toxicological evaluation of human exposure to insecticides, the following procedure was developed.

Method

Ten milliliter samples of blood were collected from each subject. Ground glass stoppered centrifuge tubes were used throughout. About 4 ml of each sample were placed in a 15-ml centrifuge tube and allowed to clot. The remaining 6 ml of blood were put in another centrifuge tube containing 0.1 ml of 20% aqueous potassium oxalate as an anticoagulant. After the contents of the tube were mixed gently by inversion, 2 ml of the oxalated whole blood were pipetted into a 15-ml centrifuge tube and held for analysis. The remaining oxalated blood was centrifuged at 2000 rpm for 10 minutes in order to separate the plasma from the red blood cells (RBC). The sample of clotted blood was centrifuged in a similar manner to remove the clot from the serum. Two ml of plasma, RBC, and serum were pipetted into separate 15-ml centrifuge tubes, and 5 ml of nanograde n-hexane were added to each of these subsamples as well as to the subsample of whole blood previously measured out. The 4 subsamples were then shaken for 3 minutes with the aid of a mini-mixer. When emulsification occurred during this extraction procedure, the emulsions were broken by centrifugation at 2000 rpm for 5 minutes. The lower aqueous phase was then removed from each tube by a capillary pipette and discarded. Each n-hexane extract was dried by adding a small amount of anhydrous Na_2SO_4 and shaking the tube on the mini-mixer. A 4-ml aliquot equivalent to 1.6 ml of each original subsample was then pipetted into a 15-ml centrifuge tube, and the tubes were placed in a 40° C constant temperature water bath. The solvent was removed with the aid of a gentle stream of clean dry air. The residue was taken up in 50 µl of nanograde hexane, and an aliquot of 10 µl equivalent to 0.22 ml of the subsample was subjected to gas liquid chromatography.

A MicroTek MT-220 gas chromatograph equipped with an electron capture detector and a strip chart recorder with a disc integrator was used. In order to confirm the identity of the materials in the effluent gas, two kinds of columns were used. Column No. 1 consisted of an aluminum tube, 6 feet x 3/16 inch, O.D., packed with 10% diethylene glycol succinate (D.E.G.S.) on 60/80 mesh, acid washed chromosorb-G. Column No. 2 consisted of an aluminum tube, 4 feet x 1/4 inch O.D., packed with 5% Dow 200 oil on 60/80 mesh acid washed chromosorb-G. Both columns were operated under the following conditions: inlet and outlet blocks 224° C; column oven, 180° C; detector, 185° C; carrier gas, N_2 80 cc/minute at 40 psi; scavenger gas, N_2 250 cc/minute at 40 psi; power source, direct current at 15 volts. The retention times in minutes for Column No. 1 and Column No. 2, respectively, were as follows: p,p'-DDT, 30.3 and 38.2; o,p'-DDT, 16.0 and 28.7; p,p'-DDE, 11.3 and 22.0; o,p'-DDE, 8.4 and 17.8; p,p'-DDD, 38.3 and 28.7; alpha-BHC, 4.4 and 4.2; beta-BHC, 23.8 and 4.6; gamma-BHC, 7.0 and 5.2; delta-BHC, 21.0 and 5.2; aldrin, 3.1 and 11.3; dieldrin, 14.4 and 22.0; and heptachlor epoxide, 9.6 and 14.4.

Results

All of the isomers and metabolites just listed except aldrin and delta-BHC have been found in the fat of persons without occupational exposure to insecticides (8,9) with the further exception that so far alpha-BHC has been reported only from India (8) and from England and Wales (9), and gamma-BHC has been reported only from England and Wales (9). Thus, it is reasonable to think that 8 isomers or metabolites of chlorinated hydrocarbon insecticides might be detectable in the blood of Americans who have no special exposure to insecticides. Actually, 6 of them (p,p'-DDT, o,p'-DDT, p,p'-DDE, beta-BHC, dieldrin, and heptachlor epoxide) were found in measurable quantity in the blood of 10 men with no occupational exposure to insecticides as shown in Table 1, which is based on results obtained with Column No. 1. Results with Column No. 2 confirmed the identity of the 6 compounds and were essentially identical from a quantitative standpoint. All of the other compounds, whether demonstrated in fat or not, would have been detected if they had been present in the subsamples of blood at a concentration of 0.0001 ppm or more.

If, as an approximation, one considers the hematocrit to be 50%, it can be readily seen that the concentration of chlorinated insecticides and related materials found in whole blood is in reasonably good agreement with the total concentrations found in plasma and RBC separately, i.e., (plasma + RBC)/2 = whole blood. As shown in Table 1, 45% or more of each n-hexane extractable insecticide and related material in the blood was found in the serum. Since there was no significant difference ($p > 0.2$) between the concentrations of insecticides or related materials found in plasma and those found in serum, most further studies were limited to analysis of serum.

TABLE 1

Concentration and Distribution of Chlorinated Insecticides and Related Materials in Ten Human Blood Samples as Determined by Electron Capture, Gas Liquid Chromatography

	p,p'-DDT	o,p'-DDT	p,p'-DDE	beta-BHC	Dieldrin	Heptachlor Epoxide
Whole Blood						
Range (ppm)	0.0024- 0.0106	0.0005- 0.0017	0.0045- 0.0235	0.0019- 0.0042	<0.0001- 0.0028	0.0004- 0.0019
Mean	0.0058	0.0010	0.0114	0.0031	0.0014	0.0008
S.E.	± 0.0009	± 0.0002	± 0.0021	± 0.0002	± 0.0002	± 0.0002
RBC						
Range (ppm)	0.0008- 0.0049	0.0002- 0.0006	0.0024- 0.0137	0.0005- 0.0022	<0.0001- 0.0009	0.0004- 0.0010
Mean	0.0026	0.0004	0.0063	0.0010	0.0005	0.0007
S.E.	± 0.0004	± 0.00004	± 0.0012	± 0.0002	± 0.0001	± 0.0001
Plasma						
Range (ppm)	0.0034- 0.0305	<0.0001- 0.0036	0.0069- 0.0499	0.0015- 0.0062	0.0009- 0.0037	0.0002- 0.0031
Mean	0.0119	0.0013	0.0257	0.0034	0.0019	0.0011
S.E.	± 0.0025	± 0.0004	± 0.0043	± 0.0005	± 0.0003	± 0.0003
Serum						
Range (ppm)	0.0042- 0.0290	0.0003- 0.0014	0.0055- 0.0475	0.0004- 0.0094	0.0003- 0.0032	0.0006- 0.0028
Mean	0.0105	0.0009	0.0198	0.0036	0.0017	0.0012
S.E.	± 0.0023	± 0.0001	± 0.0041	± 0.0008	± 0.0003	± 0.0002
Plasma vs. Serum	p>0.5	p>0.2	p>0.5	p>0.5	p>0.5	p>0.5
Proportion in Serum (%)*	90	45	87	58	61	75

$$^{*}\ \frac{1}{2} \times \frac{\text{serum}}{\text{whole blood}} \times \frac{100}{1} = \%$$

In order to determine the efficiency of the method, standard materials in amounts approximately 100 times those found in 1 ml of serum were added in n-hexane to each of six 15-ml centrifuge tubes. The factor of 100 was used so that the concentrations present in the serum subsequently added could be ignored in the calculations. The hexane was evaporated just to dryness with a gentle stream of clean dry air. To each tube was added a 1-ml aliquot of the same pooled serum, and the contents were mixed several times, using a mini-mixer, over a period of 2 hours. Three of the samples were extracted as described in the method and three samples were given three successive extractions. Data presented in Table 2 show that there were no significant differences in recovery between single and triple extractions ($p > 0.2$ in each instance) and that these recoveries ranged from a low of 29% for aldrin to a high of 91% for dieldrin.

TABLE 2

Recovery[*] of Various Chlorinated Insecticides and Related Materials Added to 1 ml Serum. Comparison of Single vs. Triple Extraction.

		Extractions			
		Single		Triple	
Material	Micrograms Added	%	S.E.	%	S.E.
p,p' - DDT	3.237	45	10.2	42	11.9
o,p' - DDT	1.082	37	9.7	37	10.0
p,p' - DDE	3.235	58	9.5	67	9.2
alpha - BHC	1.015	85	2.4	74	9.5
beta - BHC	1.058	70	2.0	66	9.9
gamma - BHC	1.029	85	2.0	73	11.0
delta - BHC	1.116	76	1.8	69	12.0
Aldrin	0.108	29	9.6	26	7.2
Dieldrin	0.098	91	5.9	76	17.3
Heptachlor epoxide	0.105	82	4.5	67	17.3

[*] Mean of three determinations.

The failure of succeeding extractions to improve recovery indicates *in vitro* interaction or binding of the added insecticides with some constituent of serum. The possibility of further *in vitro* binding during storage of the insecticides normally present in human blood was investigated. Three samples of serum were collected and aliquots taken for analysis that was completed within 2 hours. The remaining portions of the samples were stored in a refrigerator and aliquots again analyzed at the end of 5 and 7 days. There was no significant difference detected between the 3 aliquots of each sample due to storage.

The method was applied to persons who have been occupationally exposed to aldrin and dieldrin over a period of 8 years but who have not shown any clinically detectable signs of poisoning. Exposure ratings of high, medium, and low were assigned based on hours of exposure. The data presented in Table 3 show that the concentrations of aldrin and dieldrin in plasma correspond to the extent of exposure.

TABLE 3

Average Concentration of Aldrin and Dieldrin in the Plasma of Occupationally Exposed People

Exposure Rating	Mean Exposure (hours)	Persons Exposed	Aldrin (ppm)	Dieldrin (ppm)
High	6044	6	0.0023	0.0270
Medium	1410	8	0.0015	0.0206
Low	259	10	0.0007	0.0094

Plasma collected from an adult male (061229 ES 21)* about 18 hours after he ingested aldrin and about 10 hours after the last convulsion was found to contain 0.0360 ppm of aldrin and 0.279 ppm of dieldrin. Twenty days later the plasma concentration of aldrin and dieldrin had decreased to 0.0018 ppm and 0.090 ppm, respectively (10).

Plasma taken from a boy (071963 JW 24) approximately 6 hours after he swallowed lindane (99% gamma-isomer of BHC) and 3 hours after the last convulsion was found to contain gamma-BHC at a concentration of 0.29 ppm. Seven days following ingestion of lindane the patient was asymptomatic and the plasma concentration of gamma-BHC had decreased to 0.02 ppm (10).

Discussion

In order to maintain a base-line variation of less than 1% of full scale under the conditions described in this paper, the attenuation of sensitivity settings of the instrument were such that the minimal detectable amount of any of the chlorinated insecticides studied was 0.04 nanograms. This resulted in a need to concentrate the

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*Cases are identified by date of birth, first and last initials, sex, and race according to the conventions used by the AMA Council on Drugs, Registry on Adverse Reactions, for reporting adverse reactions to drugs and other chemicals.

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n-hexane extract of samples from the general population (but not from those with added material) in order to be able to inject 10 µl of extract and yet be able to report results as low as 0.0001 ppm.

With reagent blanks, the procedures used in this study did not give an elevated base line nor any peaks within the pertinent range of the chromatogram. The small amount of sodium sulfate used gave a peak only beyond the pertinent range of the chromatogram and, therefore, did not interfere with the determination of any of the compounds studied. It should be pointed out that certain plastic materials, florisil, alumina, and glass distilled water contained interfering materials that became very apparent at the sensitivity required for this procedure.

The possibility of recovering materials added to blood depends upon the manner in which they are added. If an organic solvent is added to the blood prior to addition of the standard materials or if a solution of the standard materials is added to the blood, quantitative recoveries are obtained. However, if the blood or blood fraction is added to a dry residue of the standard materials, as described in this paper, a single n-hexane extraction gives recoveries ranging from 29% for aldrin to 91% for dieldrin. Residues of all of the insecticides can be recovered quantitatively from glass in the absence of serum irrespective of the presence of water. The fact that successive extractions produced no improvement in the recovery of any of the insecticides demonstrates binding of these materials with some constituent of serum, e.g., lipoproteins. This _in vitro_ binding indicates possible _in vivo_ binding. Therefore, it may be that n-hexane extracts not the total but only the free (unbound) insecticides and related materials present in serum.

As already mentioned, it has been shown (4) that the concentration of the unbound rather than the bound foreign chemicals in plasma correlates with the intensity and duration of effect at the site of action. The concentration of each of the n-hexane extractable insecticides and related materials present in plasma (Table 3) correlates with the extent of exposure to chlorinated insecticides. Furthermore, the concentrations of dieldrin (Table 1) found in the general population by use of this method are in agreement with those obtained by Robinson (5) by use of a more lengthy procedure which was reported to give quantitative recoveries of materials that were added, albeit in solution.

Kadis and Jonasson (7) abandoned the analysis of plasma or serum for that of whole blood because insecticides are present in the red blood cell fraction also. As shown in Table 1, the relative amount of insecticides or related materials in RBC and in plasma varies depending upon the material considered. However, in all cases these materials in the blood are present in substantial concentration in the serum. It may be desirable in some studies to determine the concentration of the n-hexane extractable materials in whole blood or in each fraction of blood separately. However, for most toxicological evaluations in which the extent of exposure to insecticides is to be evaluated, the determination of the level of these materials in plasma or serum would be satisfactory.

Although no aldrin or gamma-BHC was found in any of the people of the general population, aldrin was found in the plasma of occupationally and acutely exposed persons, and gamma-BHC was found in the plasma of a boy who swallowed it. The occupationally exposed, asymptomatic persons had only 6% to 18% of the "threshold for dieldrin intoxication" (0.15 ppm) suggested by Brown *et al.* (11). The individual who swallowed aldrin had 0.036 ppm aldrin and 0.279 ppm dieldrin in his plasma at the time he had characteristic signs of poisoning. During gradual recovery, the plasma concentration of aldrin and dieldrin decreased to 0.0018 ppm and 0.090 ppm, respectively. This again correlated well with the threshold suggested by Brown *et al.* (11).

The method described has detected in blood 6 of the 8 isomers or metabolites of chlorinated hydrocarbon insecticides known to occur in the fat of people in the general population of the United States, and it has detected 2 others in people with occupational or acute exposure. Although low recoveries are obtained of added materials apparently due to binding on some constituents of blood, this may offer some advantage. In any event, the concentration of chlorinated insecticides and related materials as detected by this method follows the pattern of exposure and recovery and thus permits toxicological evaluation.

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